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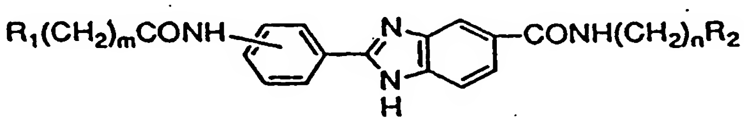
(54) **Phenylbenzimidazole derivatives**

(57) An anticancer agent, an antiviral agent or an antimicrobial agent which contains, as an active ingredient for acting on DNA, a compound presented by the following formula (1) or its pharmacologically acceptable salt:



(1)

Description

The present invention relates to compounds which are novel phenylbenzimidazole derivatives which bond to DNA and inhibit the growth of cells, and it also relates to compositions which comprise these derivatives and to the use of these derivatives for the preparation of anticancer agents, antimicrobial agents and antiviral agents.

Some of compounds which act on DNA can be used as anticancer agents. For example, adriamycin has been useful as an anticancer agent which intercalates DNA. Furthermore, compounds such as cisplatin and mitomycin which react with DNA have also often been used. Such an anticancer function based on the action on DNA can be considered to be established to some extent, though all of the function cannot be elucidated. On the other hand, in recent years, distamycin and netropsin have been noticed as substances which bond to DNA to exert an anti-tumor activity [Nature, Vol. 203, p. 1064-1065 (1964)]. Much attention has been paid to these substances as groove binders which are different from the conventional anticancer agents in a bonding mechanism to DNA. In addition, a compound such as Hoechst 33258 [Nucleic Acids Research, Vol. 16, p. 2671-2690 (1988)] is also known whose bond to DNA has already been confirmed.

However, it cannot be presently presumed at all from the knowledge regarding the conventional anticancer agents what moiety of each anticancer agent is an actually necessary structure or what other structure can be substituted. Nevertheless, it is valuable to predict and search the presence of another compound having a desirable structure. Such a search of the new structure is considered to be particularly required for the creation of a novel anticancer agent.

Moreover, compounds in which an alkylating agent is bonded to the distamycin derivatives are known, and typical examples of such compounds are described in J. Am. Chem. Soc., Vol. 107, p. 8266 (1985), EP-A-246868, WO 93-13739, J. Med. Chem., Vol. 32, p. 774 (1989), and the like. Other compounds are also known in which an alkylating agent is bonded to a compound similar to the distamycin (US-A-5273991). In several of these compounds, a bis(2-chloroethyl)amino residue is used as the alkylating agent, but it is already known that this residue is a part of the structure of the anticancer agent. For example, chlorambucil is known as the anticancer agent having the bis(2-chloroethyl)amino residue in its molecule. The anticancer activity of this compound is presumed to be the result of its alkylation to DNA, an enzyme or the like. However, the merit of adding the alkylating agent having a chloroethylamine structure as a partial structure of the anticancer agent which bonds to DNA has scarcely been recognized so far.

The above-mentioned distamycin is a typical example of the compounds which bond to DNA, but compounds which have benzimidazole in each molecule and which can bond to DNA are also known. For example, Hoechst 33258 has a structure containing two benzimidazoles, a phenol and a piperazine. In this compound, however, a feature that aromatic rings are bonded via amide bonds as in the distamycin is not present. Comparing these structures with each other, the structures which can bond to DNA are definitely different from each other. That is to say, the structures capable of bonding to DNA can be classified into some groups, but there has not been found any report in which the evaluation of their usefulness is made.

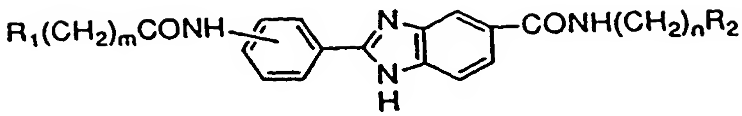
The present inventors have searched a novel structure in expectation of the presence of a compound which has a structure other than known compounds, partially maintaining the structure of benzimidazole and which has an anticancer function. As a result, novel compounds having a structure, in which a phenyl group is directly bonded to benzimidazole and another substituent is added via an amide bond, have been selected as useful anticancer agents. Above all, 1H-2-phenylbenzimidazole-5-carboxamide derivatives have been particularly selected. These compounds possess novel structures. Furthermore, these compounds show an inhibitory activity on tumor cell growth equal to that of distamycin in vitro. This activity as the anticancer agent has also been newly found by the present inventors.

The activity as the anticancer agent can probably be enhanced by the addition of an alkylating agent. This has been simultaneously investigated, and as a result, it has been apparent that a compound containing the alkylating agent exerts a higher anti-tumor activity as compared with another compound containing no alkylating agent. It has been understood from this fact that the compound having the skeleton of phenylbenzimidazole can become a high-active anticancer agent by adding the alkylating agent into the molecule of the compound.

On the basis of the above-mentioned knowledge, the present inventors have completed the invention of novel anti-cancer agents.

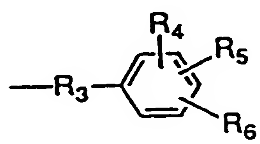
An object of the present invention is to provide a novel compound which acts on DNA, or a novel compound which has a partial structure capable of acting on DNA and which is useful as an anticancer agent.

A compound of the present invention which can achieve the above-mentioned object is a compound represented by the following formula (1) or its pharmacologically acceptable salt:



(1)

wherein each of m and n is independently an integer of from 0 to 5; each of R_1 and R_2 is independently a hydrogen atom, a halogen atom, an alkylthio group having 1 to 8 carbon atoms, preferably 1 to 4 carbon atoms, an amino group which may be substituted, an ammonium group which may be substituted, a sulfonium group which may be substituted, a phenyl group which may be substituted, a hetero-five-membered ring group which may be substituted, a hetero-six-membered ring group which may be substituted, an amidino group, a guanidino group, an amino acid residue or a group represented by the formula (2)



(2)

wherein R_3 is a direct bond or an oxygen atom {when R_3 is an oxygen atom, m or n of $(CH_2)_m$ or $(CH_2)_n$ to which R_3 bonds is not 0}; R_4 is a hydrogen atom, an alkyl group having 1 to 8 carbon atoms, an alkoxy group having 1 to 8 carbon atoms, a halogen atom, a trifluoromethyl group, a cyano group, an amidino group, a carboxyl group or $-COR_7$ wherein R_7 is an alkylamino group having 1 to 8 carbon atoms which may be substituted by a substituted amino group, an amino group which may be substituted by a phenyl group which may be substituted, or a benzylamino group which may be substituted; R_5 is a hydrogen atom, an alkyl group having 1 to 8 carbon atoms, an alkoxy group having 1 to 8 carbon atoms or a halogen atom; R_6 is a $-(CH_2)_pN(R_8)_2$ or $-(CH_2)_pNR_8R_9$ wherein p is an integer of from 0 to 5; R_8 is $-CH_2CH_2W$ wherein W is a halogen atom, a hydroxyl group, a mesyloxy group or a tosyloxy group or $-OCOR_7$ wherein R_7 is as defined above; R_9 is an alkyl group having 1 to 5 carbon atoms or a mesyl group; and the phenyl group in formula (1) having a $R_1(CH_2)_mCONH$ group can be substituted by the $R_1(CH_2)_mCONH$ group at any position, preferably at the 3-position or the 4-position of the phenyl group.

The compound of the present invention acts on DNA, and so it is useful as an active ingredient of an anticancer agent, an antiviral agent or an antimicrobial agent.

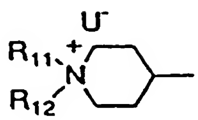
Now, the present invention will be described in more detail.

In formula (1), examples of "a halogen atom" represented by R_1 or R_2 include Cl, Br and I.

Examples of "an amino group which may be substituted" represented by R_1 or R_2 include an amino group, monoalkylamino groups and dialkylamino groups substituted by a straight-chain or a branched alkyl group having 1 to 8 carbon atoms. As the dialkylamino groups, those having the alkyl groups of 1 to 4 carbon atoms are desirable. Above all, a methylamino group, ethylamino group, n-propylamino group, isopropylamino group, n-butylamino group, dimethylamino group, diethylamino group, dipropylamino group and diisopropylamino group are desirable.

Examples of "an alkylthio group having 1 to 8 carbon atoms" represented by R_1 or R_2 include straight-chain and branched alkylthio groups having 1 to 8 carbon atoms, and typical suitable examples thereof include a methylthio group, ethylthio group, n-propylthio group, isopropylthio group, n-butylthio group, isobutylthio group, t-butylthio group, n-pentylthio group, n-hexylthio group, n-heptylthio group and n-octylthio group.

Examples of "an ammonium group which may be substituted" represented by R_1 or R_2 include a trimethylammonium group, a triethylammonium group and ammonium groups represented by the following formulae (3-1) to (3-14):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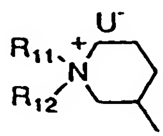
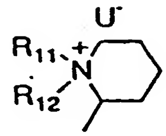

(3-2)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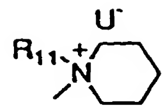
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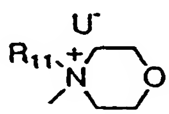
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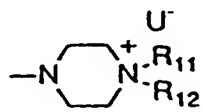
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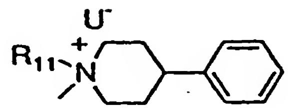
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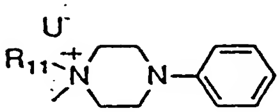
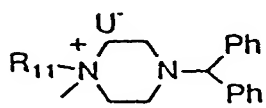
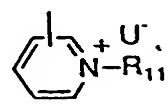

(3-8)


(3-9)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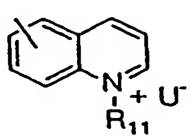
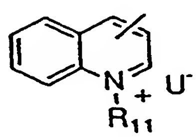

(3-11)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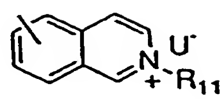
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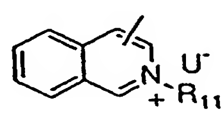
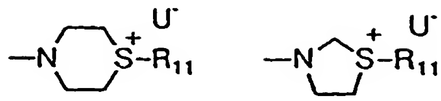

(3-14)

The acid residue found as a counter ion to the eve "ammonium group which may be substituted" is required to be usable as a portion of drug, and examples of the acid residue include inorganic acid residues for example derived from hydrogen chloride, hydrogen iodide, hydrogen bromide, tetrafluoroboric acid, perchloric acid and phosphoric acid, organic sulfonic acid residues such as methanesulfonic acid, toluenesulfonic acid, camphorsulfonic acid and 1,5-naphthalenedisulfonic acid, and carboxylic acids such as lactic acid, maleic acid and malonic acid. In these formulae, each of R_{11} and R_{12}, is independently a straight-chain or a branched alkyl group having 1 to 8 carbon atoms, and suitable examples thereof include a methyl group, ethyl group, n-propyl group, isopropyl group, n-butyl group, isobutyl group, t-butyl group, n-pentyl group, n-hexyl group, n-heptyl group and n-octyl group.

Examples of "a sulfonium group which may be substituted" represented by R_1 or R_2 include sulfonium groups substituted by a straight-chain or a branched alkyl group having 1 to 3 carbon atoms, and typical examples thereof include a dimethylsulfonium group, diethylsulfonium group, methylethylsulfonium group, methylpropylsulfonium group, diisopropylsulfonium group, methylisopropylsulfonium group, and sulfonium groups represented by the formulae (4-1) or (4-2):



(4-1) (4-2)

The acid residue bound to the above "sulfonium group which may be substituted" is required to be usable as a portion of drug, and examples of the acid residue include inorganic acid residues such as hydrogen chloride, hydrogen iodide, hydrogen bromide, tetrafluoroboric acid, perchloric acid and phosphoric acid, organic sulfonic acid residues such as methanesulfonic acid, toluenesulfonic acid, camphorsulfonic acid and 1,5-naphthalenedisulfonic acid, and carboxylic acids such as lactic acid, maleic acid and malonic acid. In these formulae, R_{11} is a straight-chain or a branched alkyl group having 1 to 8 carbon atoms, and suitable examples thereof include a methyl group, ethyl group, n-propyl group, isopropyl group, n-butyl group, isobutyl group, t-butyl group, n-pentyl group, n-hexyl group, n-heptyl group and n-octyl group.

Examples of "a phenyl group which may be substituted" represented by R_1 or R_2 include a phenyl group which may be substituted with at least one of halogen atoms (a fluorine atom, a chlorine atom, a bromine atom and an iodine atom), straight-chain and branched alkyl groups having 1 to 5 carbon atoms, straight-chain and branched alkoxy groups having 1 to 3 carbon atoms, alkoxycarbonyl groups having 2 to 4 carbon atoms, a trifluoromethyl group, a cyano group, an amidino group, a guanidino group and dialkylamino groups in which the alkyl groups have 1 to 3 carbon atoms, respectively. Suitable examples thereof include a chlorophenyl group, dichlorophenyl group, trichlorophenyl group, bromophenyl group, dibromophenyl group, tribromophenyl group, fluorophenyl group, difluorophenyl group, trifluorophenyl group, methylphenyl group, ethylphenyl group, n-propylphenyl group, isopropylphenyl group, n-butylphenyl group, isobutylphenyl group, t-butylphenyl group, n-pentylphenyl group, methoxyphenyl group, ethoxyphenyl group, n-propyloxyphenyl group, isopropyloxyphenyl group, methoxycarbonylphenyl group, ethoxycarbonylphenyl group, n-propyloxycarbonylphenyl group, trifluoromethylphenyl group, cyanophenyl group, amidinophenyl group, guanidinophenyl group, dimethylaminophenyl group, diethylaminophenyl group, dipropylaminophenyl group, methoxyphenyl group and 3,4,5-trimethoxy group. In the case of monosubstition, the position of the substituent on the phenyl group is the 2-, 3- or 4-position; in the case of disubstitution, the positions of the substituents thereon are two positions of the 2-, 3-, 4-, 5- and 6-positions; and in the case of trisubstitution, the positions of the substituents thereon are three positions of the 2-, 3-, 4-, 5- and 6-positions, unless otherwise noted.

Examples of "a hetero-five-membered ring group which may be substituted" represented by R_1 or R_2 include a pyrrolyl group, furyl group, thienyl group, imidazolyl group, oxazolyl group, pyrazolyl group, thiazolyl group, isothiazolyl group, isoxazolyl group, pyrrolinyl group, imidazolidinyl group, pyrazolidinyl group, pyrazolinyl group, furazanyl group, tetrahydrofuranyl group, triazolyl group and tetrazoyl group.

Examples of "a hetero-six-membered ring group which may be substituted" represented by R_1 or R_2 include a pyridyl group, pyrimidinyl group, pyranyl group, pyrazinyl group, pyridazinyl group, piperidyl group, piperazinyl group, thiomorpholino group, 4-methyl-1-piperazino group, 4-benzyl-1-piperazino group, 1-morpholino group, 1-piperidino group, 4-piperidino group and 4-methyl-1-piperidino group.

Here, in the above-mentioned "a hetero-five-membered ring group which may be substituted" and "a hetero-six-membered ring group which may be substituted", the passage "may be substituted" means that this group may be substituted by, for example, a halogen atom (a fluorine atom, a chlorine atom, a bromine atom or an iodine atom), a straight-chain and branched alkyl group having 1 to 5 carbon atoms, a straight-chain and branched alkoxy group having 1 to 5 carbon atoms, an alkoxycarbonyl group having 2 to 4 carbon atoms, a haloalkyl group having 1 to 3 carbon atoms, a cyano group, an amidino group and/or a dialkylamino group having 1 to 3 carbon atoms.

"An amino acid residue" as R_1 or R_2 is defined specially in the present invention as a group which can be obtained by omitting a carboxyl group from an amino acide. Suitable examples of this amino acid include arginine, histidine and lysine.

"A direct bond" represented by R_3 means that the substituted or non-substituted phenyl group in the formula (2) is directly bonded via no R_3.

Examples of a halogen atom represented by R_4 or R_5 in the formula (2) include F, Cl, Br and I.

Suitable examples of a halogen atom of W in $-CH_2CH_2W$ represented by R_8 include Cl and Br.

Preferable examples of an alkyl group having 1 to 8 carbon atoms represented by R_4 or R_5 include a methyl group, ethyl group, n-propyl group, isopropyl group, n-butyl group, isobutyl group, t-butyl group, n-pentyl group, n-hexyl group, n-heptyl group and n-octyl group.

Preferable examples of an alkoxy group having 1 to 8 carbon atoms represented by R_4 or R_5 include a methoxy group, ethoxy group, n-propyloxy group, isopropyloxy group, n-butoxy group, isobutoxy group, t-butoxy group, n-pentyloxy group, n-hexyloxy group, n-heptyloxy group and n-octyloxy group.

Preferable examples of "an alkylamino group which may be substituted by a substituted amino group" represented by R_7 include a dimethylaminoethyl group, dimethylaminopropyl group, dimethylaminobutyl group, diethylaminoethyl group, diethylaminopropyl group, diethylaminobutyl group, dipropylaminoethyl group, dipropylaminopropyl group, dipropylaminobutyl group, diisopropylaminoethyl group, diisopropylaminopropyl group and diisopropylaminobutyl group.

Preferable examples of "an amino group which may be substituted by a phenyl group which may be substituted" represented by R_7 include aniline, 4-dimethylaminoaniline and 4-chloroaniline.

Preferable examples of "a benzylamino group which may be substituted" represented by R_7 include benzylamine and 4-dimethylaminobenzylamine.

As a pharmacologically acceptable salt, any salt is acceptable, so far as it is usable as a drug, and examples of the pharmacologically acceptable salt include inorganic acid salts such as hydrochlorides, hydrobromates, hydroiodates, sulfates, nitrates and phosphates, and organic acid salts such as methanesulfonates, toluenesulfonates, camphorsulfonates, acetates, fumarates, maleates, citrates, oxalates and tartrates.

Next, the compounds represented by formula (1) will be classified into groups A and B, and typical preparation processes thereof will be described.

Group A: A compound in which R_1 is a hydrogen atom, a halogen atom or a group represented by formula (2), or its pharmacologically acceptable salt.

Group B: A compound in which R_2 is a hydrogen atom, a halogen atom or a group represented by formula (2), or its pharmacologically acceptable salt.

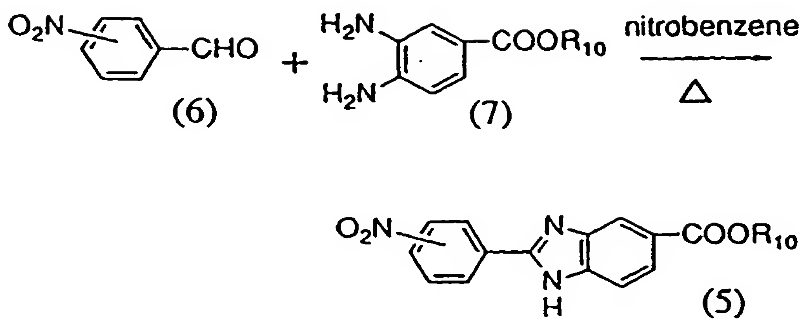
In the undermentioned description and examples, the following abbreviations represent the corresponding compounds:

"DCC" ... N,N'-dicylohexylcarbodiimide,
"CDI" ... N,N'-carbonyldiimidazole,
"HOSu" ... N-hydroxysuccinimide,
"EDCI" ... 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride,
"DECP" ... Diethyl cyanophosphonate,
"HOBt" ... 1-hydroxybenzotriazole,
"DMAP" ... 4-dimethylaminopyridine,
"DMF" ... Dimethylformamide,
"THF" ... Tetrahydrofuran,
"DMSO" ... Dimethyl sulfoxide,
"IPA" ... Isopropanol,
"chlorambusyl" ... 4-[4-{bis(2-chloroethyl)amino}phenyl]butyric acid, and
"Pd/C" ... Active carbon with palladium (usually, the palladium content is 5-10%).

I. Synthesis of an intermediate

As shown in the following reaction formula (1), an aldehyde of formula (6) and 3,4-diaminobenzoic acid or its ester of formula (7) are heated at a temperature of from 100°C to a reflux temperature, preferably at 130 to 200°C in nitrobenzene, and the temperature of the reaction system is then returned to room temperature. A produced 1H-2-phenylbenzimidazole-5-carboxylic acid derivative or its ester derivative represented by formula (5) can be collected by filtration:

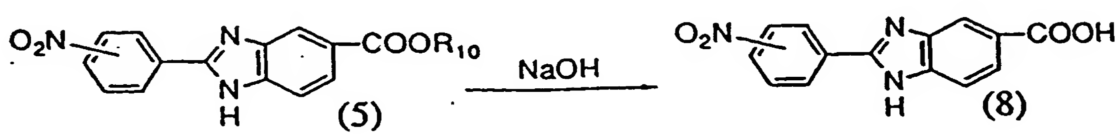
Reaction formula (1):



Reaction formula (1)

Here, when the ester is obtained, this ester can be hydrolyzed as shown in the following reaction formula (2), thereby obtaining a 1H-2-phenylbenzimidazole-5-carboxylic acid derivative represented by formula (8):

Reaction formula (2):



Reaction formula (2)

No particular restriction is put on the amounts of the aldehyde of formula (6) and the 3,4-diaminobenzoic acid ester of formula (7), but in general, the amount of the latter is in the range of from 80 to 120 mol parts with respect to 100 mol parts of the former. The reaction in nitrobenzene is carried out by heating, until the starting materials have disappeared, while the progress of the reaction is observed, but in either case, a heating time of 5 to 100 hours leads to successful results. The heating may be carried out continuously or intermittently, but the total heating time should be in the above-mentioned range. The hydrolysis of the ester can be accomplished by heating the ester in the presence of sodium hydroxide or potassium hydroxide in a mixed solvent of water and ethanol or water and methanol. In this case, the ratio of water is in the range of from 5 to 90% by weight, preferably from 40 to 60% by weight.

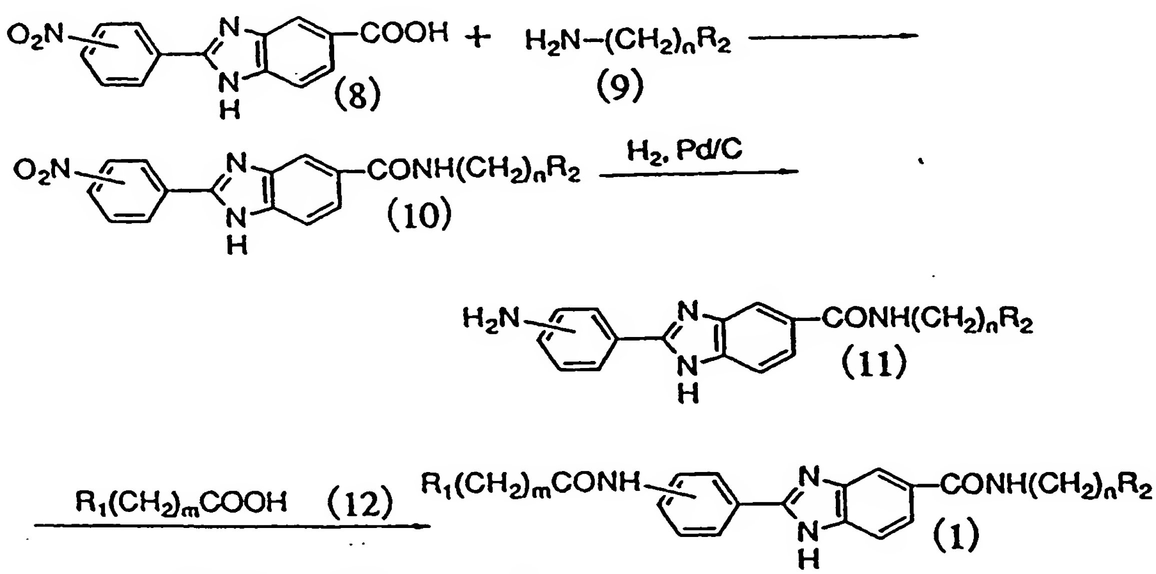
The heating temperature is in the range of from 50°C to a reflux temperature.

Concretely, if 3-nitrobenzaldehyde or 4-nitrobenzaldehyde is selected as the starting material, 1H-2-(3-nitrophenyl)benzimidazole-5-carboxylic acid or 1H-2-(4-nitrophenyl)benzimidazole-5-carboxylic acid can be synthesized by the same procedure as described above. The compound represented by the formula (8) can be used for the synthesis of compounds in the groups A and B.

II. Synthesis of a compound in the group A

As shown in the following reaction formula (3), for example, an amino compound represented by formula (9) having R_2 [which is as defined above in the case of the formula (1)] is bonded to a carboxylic acid represented by formula (8) in the presence of a suitable condensing agent such as DCC, CDI, EDCI or DECP to obtain an intermediate of formula (10). Next, the nitro group of this intermediate is reduced to a corresponding amino compound of formula (11) by catalytic hydrogenation, and this amino compound is then reacted with a carbonyl compound represented by formula (12) having R_1 [which is a hydrogen atom, a halogen atom or a group represented by formula (2)] to synthesize a compound of formula (1) in the group A:

Reaction formula (3):



Reaction formula (3)

As a solvent for use in the bonding of the amino compound of formula (9) and the carboxylic acid of formula (8) in the presence of the condensing agent, various kinds of solvents are usable, but DMF or a mixed solvent containing DMF is preferred.

The reaction is preferably carried out in the range of from -5 to 30°C. During the reaction, the progress of the reaction can be observed, but a reaction time is usually in the range of from 1 to 50 hours.

As the amino compound represented by formula (9) having R_2, there can be used a compound synthesized from known compounds by combining known reactions, or a commercially available reagent. For example, when dimethylaminopropylamine, diethylaminopropylamine or dipropylaminopropylamine is used as the compound of formula (9), a product can be obtained in which R_2 is a substituted amino group. Alternatively, when methylthiopropylamine, ethylthiopropylamine, propylthiopropylamine or butylthiopropylamine is used, an alkylthio group can be introduced into R_2. Furthermore, when aminopropyltrimethylammonium or aminopropyltriethylammonium is used, a trimethylammonium group or a triethylammonium group can be introduced into R_2. The phenyl group which may be substituted, the hetero-five-membered ring group which may be substituted, or the hetero-six-membered ring group which may be substituted can be introduced into R_2 of the compound in the group A by using the compound of formula (9) in which R_2 is the group to be introduced.

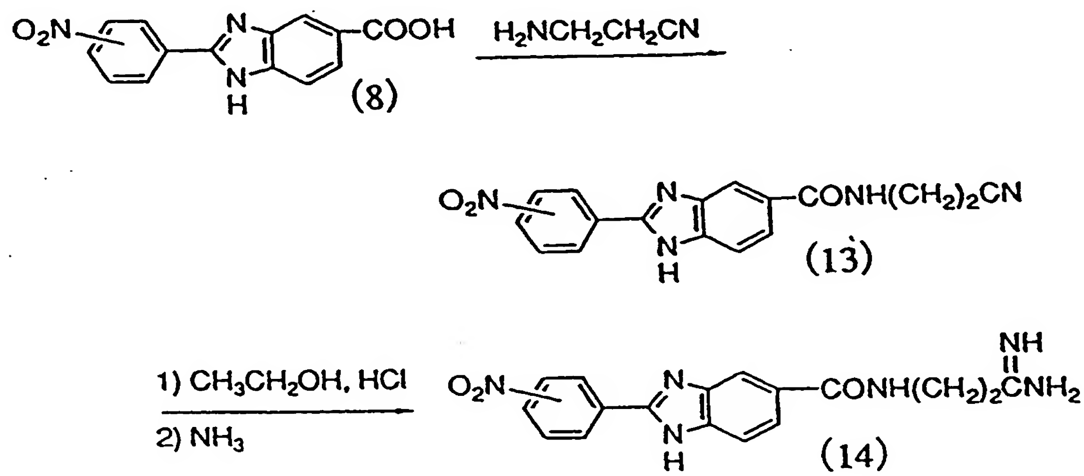
A compound in which R_2 is an amidino group can be synthesized as follows.

As shown in the following reaction formula (4), the carboxylic acid of formula (8) is first reacted with β-aminopropionitrile to obtain a reaction mixture containing a compound of formula (13).

At this time, a usual condensing agent such as DCC, CDI, EDCI or DECP can be used. A method in which HOBt or HOSu is added to DCC can also be used. The reaction is suitably carried out at 0 to 30°C.

Next, this reaction mixture is suspended in ethanol, and a hydrogen chloride gas is then fed. Afterward, the resultant crystal is collected by filtration, and then dissolved or suspended in a solvent. Ammonia gas is further introduced into the solution to obtain a desired amidino compound represented by formula (14). In this case, the solvent is preferably ethanol or a mixed solvent of ethanol and methanol. In the mixed solvent, the ratio of ethanol can optionally be selected in the range of from 10 to 100%. If this amidino compound is used as the compound of formula (10) in reaction formula (3), the compound of the group A in which R_2 is an amidino group can be obtained:

Reaction formula (4):



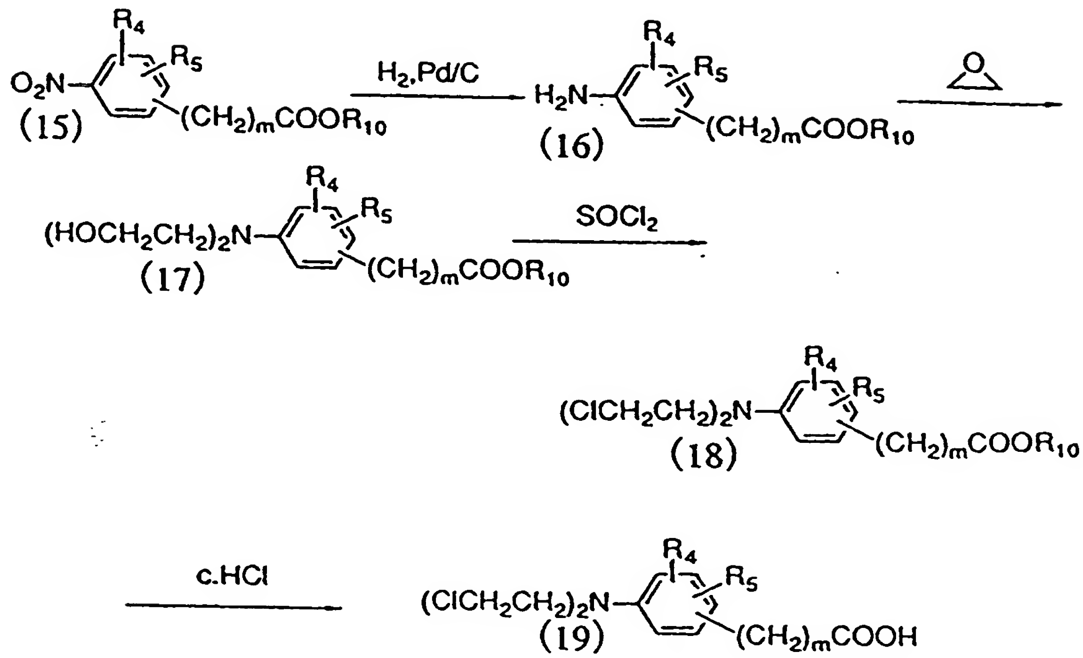
Reaction formula (4)

Typical examples of the compound of formula (8) in the reaction formulae (3) and (4) include 1H-2-(3-nitrophenyl)benzimidazole-5-carboxylic acid or 1H-2-(4-nitrophenyl)benzimidazole-5-carboxylic acid.

Next, reference will be made to a synthesis method of a compound in which R_1 is a desired group.

For example, a compound in which R_1 is a substituent represented by formula (2) can be synthesized by a process shown by the following reaction formula (5):

Reaction formula (5):



Reaction formula (5)

In the first place, a carboxylic acid ester derivative of formula (15) having a nitro group is reduced to a corresponding amino compound of formula (16) by catalytic hydrogenation using Pd/C as a catalyst. At this time, methanol, ethanol, DMF and the like can be used singly or in the form of a mixture of two or more thereof as a solvent. The reaction is preferably carried out at a temperature of from 0 to 30°C. A reaction time is in the range of from 30 minutes to 2 hours.

As the carboxylic acid derivative represented by formula (15) which can be used herein, there can be used a compound synthesized from known compounds by known reactions, or a commercially available reagent.

Next, the thus produced amino compound of formula (16) is reacted with ethylene oxide to obtain a compound of formula (17). At this time, a mixture obtained by mixing a solvent such as water, THF, dichloromethane or benzene and acetic acid at an optional ratio can be used as a solvent. The reaction is preferably carried out at a temperature of from -20 to 120°C. A reaction time is suitably in the range of from 1 to 50 hours.

Furthermore, the OH group of the compound of formula (17) is replaced with a Cl group by the use of a suitable reagent such as,thionyl chloride, oxalyl chloride, phosphorus pentachloride, phosphorus oxychloride, mesyl chloride (in DMF), a combination of mesyl chloride and sodium chloride, a combination of mesyl chloride and lithium chloride, or dichlorotriphenylphosphorane, thereby obtaining a compound of formula (18). The reaction is carried out at a temperature of from 0 to 100°C. The reaction time is in the range of from 20 minutes to 5 hours. In this case, a usual solvent such as chloroform, benzene or toluene can be used. Moreover, this solvent can be mixed with DMF. When thionyl chloride or oxalyl chloride is used, the reaction can be carried out without solvent.

The compound represented by formula (18) can be hydrolyzed with an acid to obtain a carboxylic acid derivative represented by formula (19). For example, the hydrolysis can be successfully accomplished with concentrated hydrochloric acid by heating at a temperature of from 80°C to a reflux temperature. The reaction time is suitably in the range of from 30 minutes to 5 hours.

When this compound of formula (19) is used as the compound of formula (12) in the reaction formula (3), the compound of the group A in which R_1 is a substituent represented by formula (2) can be obtained.

For example, the compound represented by formula (11) can be bonded to the compound represented by formula (19) by the use of a suitable condensing agent such as DCC, CDI, EDCI or DECP. In this case, a usual solvent is usable, but DMF or a mixed solvent containing DMF is preferred. The reaction is preferably carried out in the range of from -5

to 30°C. During the reaction, the progress of the reaction can be observed, but the reaction time is usually in the range of from 1 to 50 hours. Alternatively, the compound of formula (19) can be treated with thionyl chloride or oxalyl chloride in a usual solvent (e.g., methylene chloride, chloroform, toluene and DMF can be used singly or in the form of a mixture of two or more thereof) to obtain an acid chloride, and this product can be then reacted with the compound represented by formula (11) to bond them to each other. Here, the reaction is preferably carried out in the range of from -5 to 30°C. During the reaction, the progress of the reaction can be observed, but the reaction time is usually in the range of from 1 to 50 hours.

Another side chain represented by R_1 can also be prepared by using commercially available reagents and several steps of known reactions.

III. Synthesis of a compound in the group B

First, a technique of introducing R_2 will be described.

To start with, reference will be made to the synthesis of a compound in which R_2 is, for example, a substituent represented by formula (2).

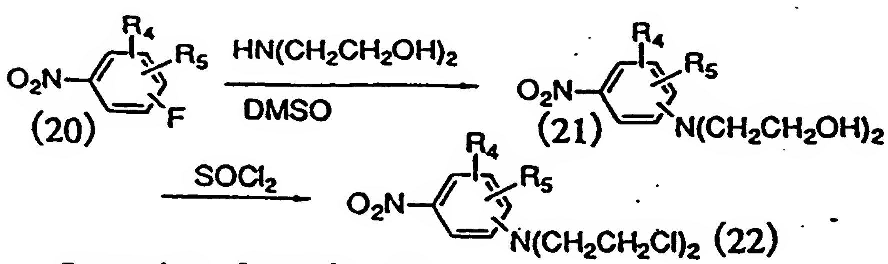
The introduction of R_6 into the substituent represented by formula (2) can usually be achieved by each of the following two methods.

Method A:

As shown in the following reaction formula (6), a halogenated benzene derivative (in formula (20), F is shown as an example of a halogen atom) containing a nitro group and suitable substituents which is represented by formula (20) is reacted with N,N-bis(2-hydroxyethyl)amine to obtain a compound of formula (21). In this time, DMSO is used as a solvent, and the reaction temperature is in the range of from 20 to 150°C. The reaction time is preferably in the range of from 30 minutes to 10 hours. As the halogenated benzene derivative containing the suitable substituents, there can be used a commercially available reagent or a compound which can be synthesized by the use of a known reaction. For example, the halogenated benzene derivative containing an amidino group can be introduced from a halogenated benzene derivative containing a cyano group by the utilization of a known reaction.

Next, the thus obtained intermediate of formula (21) is reacted with a suitable chlorinating agent such as thionyl chloride, oxalyl chloride, phosphorus pentachloride, phosphorus oxychloride, mesyl chloride or a combination of mesyl chloride (in DMF) and sodium chloride to obtain a chloride of formula (22). The reaction is carried out at 0 to 150°C, and a reaction time is in the range of from 5 minutes to 5 hours. As a solvent, there can be used a usual solvent such as chloroform, benzene or toluene. Furthermore, such a solvent can be mixed with DMF. In addition, the reaction can be carried out without solvent.

Reaction (6):



Reaction formula (6)

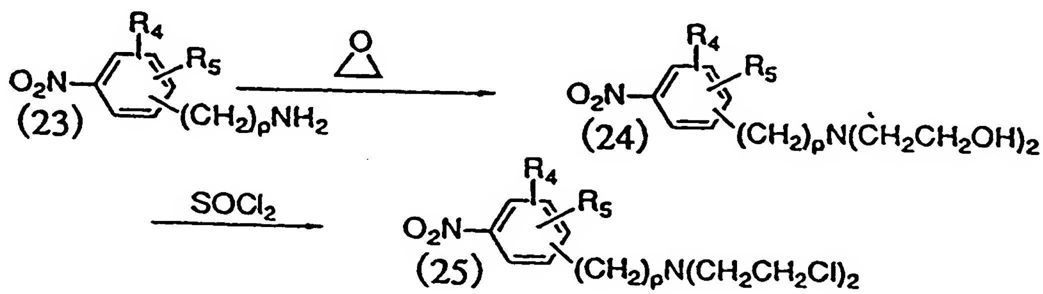
Method B:

As shown in the following reaction formula (7), an aniline derivative (in the case that p is 0) or an aminoalkylbenzene derivative (in the case that p is 1 or more) having a nitro group and suitable substituents which is represented by formula (23) is reacted with ethylene oxide to obtain a compound of formula (24). As a solvent at this time, there can be used a

mixture obtained by mixing a solvent such as water, THF, dichloromethane or benzene with acetic acid at an optional ratio. The reaction is suitably carried out at -20 to 120°C, and a reaction time is suitably in the range of from 1 to 50 hours. As the aniline derivative or the aminoalkylbenzene derivative having the nitro group and the suitable substituents, there can be used a commercially available reagent or a compound which can be synthesized by the use of a known reaction.

Next, the compound of formula (24) can be chlorinated with a suitable chlorinating agent such as thionyl chloride, oxalyl chloride, phosphorus pentachloride, phosphorus oxychloride, mesyl chloride (in DMF) or a combination of mesyl chloride and sodium chloride to obtain a compound of formula (25). The reaction is suitably carried out at 0 to 150°C, and the reaction time is in the range of from 5 minutes to 5 hours. As a solvent, there can be used a usual solvent such as chloroform, benzene or toluene. Furthermore, such a solvent can be mixed with DMF. In addition, the reaction can be carried out without solvent in the case of thionyl chloride or oxalyl chloride.

Reaction (7):

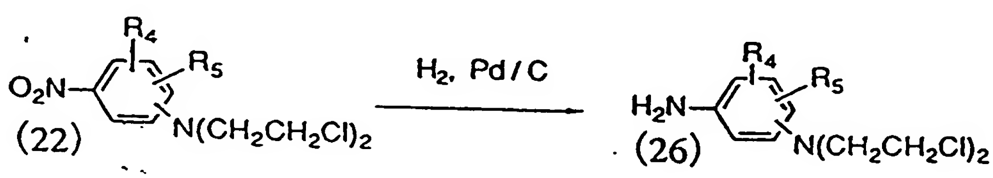


Reaction formula (7)

Each of the compounds of formulae (22) and (25) obtained in the above-mentioned methods A and B can be further subjected to the following reaction to introduce the substituent represented by formula (2) into the compound in the group B.

In the first place, each of these compounds (nitro compounds) is catalytically hydrogenated to obtain a corresponding amino compound. The reaction formula in which the compound of formula (22) is used is as follows.

Reaction formula (8):



Reaction formula (8)

The nitro compound of formula (25) can also be converted into the amino compound in the same manner.

When hydrochloric acid is added in an amount equal to or more than mols of the nitro compound, usually in an amount of from 1 to 1.2 mols at the catalytic hydrogenation, the amino compound can be stably obtained. The catalytic hydrogenation is suitably carried out at 5 to 30°C, and a usual solvent can be used. For example, DMF or a mixed solvent of DMF and methanol is desirable. The ratio of DMF in this mixed solvent can be selected in the range of from 5 to 100%, preferably from 20 to 100%.

For the synthesis of the corresponding amino compound from the usual nitrobenzene derivative, there is usually employed the catalytic hydrogenation using Pd/C as a catalyst or a process utilizing a reaction in which tin chloride and hydrochloric acid are used. In particular, as the reduction method of the above-mentioned nitrobenzene derivative to which N,N-bis(2-chloroethyl)amino group is bonded, there is known a reduction method using tin chloride and hydrochloric acid, as reported in, for example, J. Chem. Soc. p. 1972-1983 (1949) or J. Med. Chem. Vol. 33, p. 112 to 121 (1990).

However, in place of this usual method, the following technique can also be used to efficiently perform the reduction reaction, and some treatments subsequent to the reaction can easily be accomplished advantageously. That is to say, the nitro compound which is the starting material is dissolved in a suitable solvent, for example, a single solvent or a mixed solvent of ethanol, methanol, ethyl acetate, THF and DMF, and Pd/C is then added in an amount corresponding to 0.5 to 50% by weight of the nitro compound, followed by hydrogenation at room temperature under atmospheric pressure, to obtain the corresponding amino compound. At this time, hydrochloric acid can be added in an amount equal to or more than mols of the nitro compound, usually in an amount of from 1 to 1.2 mols. The catalyst is removed by filtration and the solvent is then distilled off, followed by a treatment with one or more kinds of suitable solvents such as ethanol, IPA and ether, whereby a desired hydrochloride can be simply obtained.

Furthermore, as shown in the following reaction formula (9), an amino compound of formula (28) can be synthesized from a carboxylic acid of formula (27) in accordance with a process shown in J. Med. Chem. Vol. 33, p. 3014-3019 (1990).

Reaction formula (9):

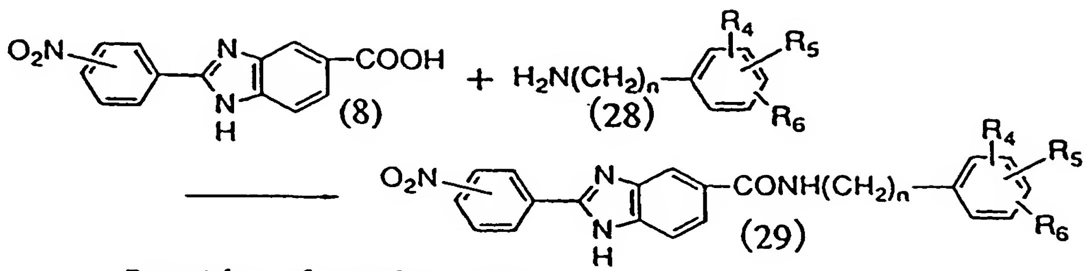
Reaction formula (9)

By the use of this process, an aminoalkylbenzene derivative can be synthesized in which R_3 is a chain having two or more methylene groups.

Here, the compound of formula (26) corresponds to the compound of formula (28) in which n is 0 and R_6 is an N,N-bis(2-chloroethyl)amino group. In the following description, therefore, reactions using the compound having formula (28) will be referred to, but needless to say, the compound of formula (26) can be used as the compound of formula (28).

As shown in the reaction formula (10), a benzimidazole derivative of formula (8) is bonded to the amino compound of formula (28) in the presence of a usual condensing agent (CDI, DECP, DCC, a combination of DCC and HOBt, or the like) to synthesize the compound of formula (29).

Reaction formula (10):

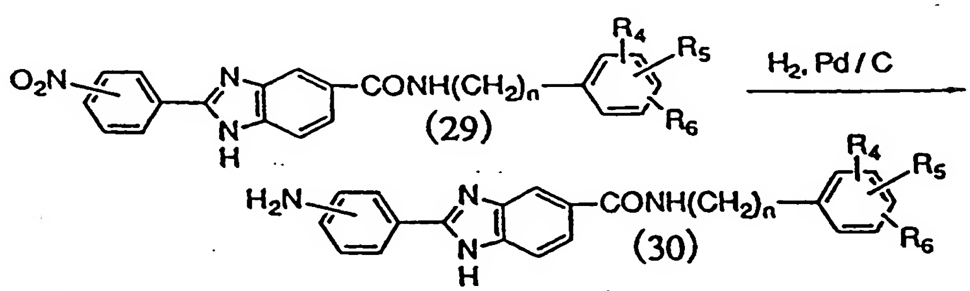


Reaction formula (10)

As a solvent for this reaction, DMF is desirable, but another usual solvent can also be used. The state of this reaction can be observed by TLC or the like to confirm the completion of the reaction, but the reaction is preferably carried out for a period of from 1 to 40 hours. The reaction temperature is preferably in the range of from -5 to 40°C.

Moreover, as shown in the following reaction formula (11), the nitro group of the compound of formula (29) can be reduced to a corresponding amino group by catalytic hydrogenation using Pd/C as a catalyst.

Reaction formula (11):



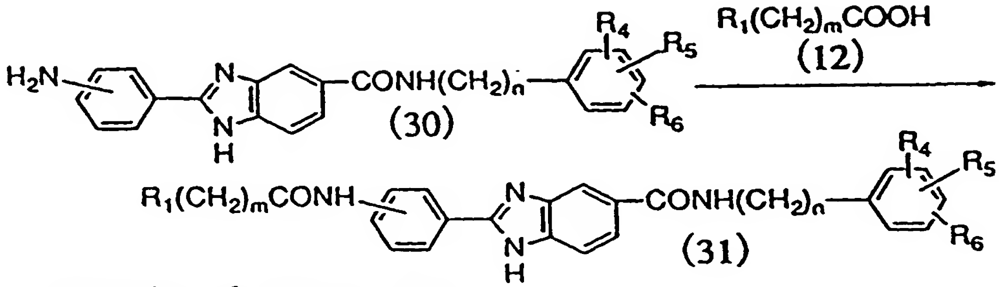
Reaction formula (11)

This reaction proceeds substantially quantitatively. In this case, ethanol, methanol and DMF can be used singly or in a combination of two or more thereof as a solvent. At this time, hydrochloric acid can be added in an amount equal to or more than mols of the nitro compound, usually in an amount of from 1 to 1.2 mols. The reaction is preferably carried out at a temperature of from 0 to 40°C for a period of from 10 minutes to 20 hours.

The bonding of the aniline derivative in the reaction formula (10) and the successive reducing reaction in the reaction formula (11) establish a novel synthetic route which has not been found in literature so far. For example, a bonding reaction of a distamycin derivative and N,N-bis(2-chloroethyl)-1,4-phenylenediamine is disclosed in Japanese Patent Laid-open No. 92933/1994, but in this publication, a distamycin moiety is previously synthesized, and the bonding reaction of N,N-bis(2-chloroethyl)-1,4-phenylenediamine is finally carried out. This is based on a conception that the bonding of an N,N-bis(2-chloroethyl)amino group which is highly chemically reactive is performed in the last step. On the contrary, the present inventors have developed the technique that even if the aniline derivative moiety is previously introduced into the molecule, the desired compound can be obtained in a high yield.

Next, as shown in the following reaction formula (12), the amino compound of formula (30) obtained by the previous reducing reaction is bonded to the carboxylic acid derivative of formula (12) in the presence of a usual condensing agent such as DCC, CDI, EDCI or DECP to introduce a R_1 moiety to the amino compound, thereby obtaining a compound in the group B [R_2 is a group shown in formula (2)].

Reaction formula (12):



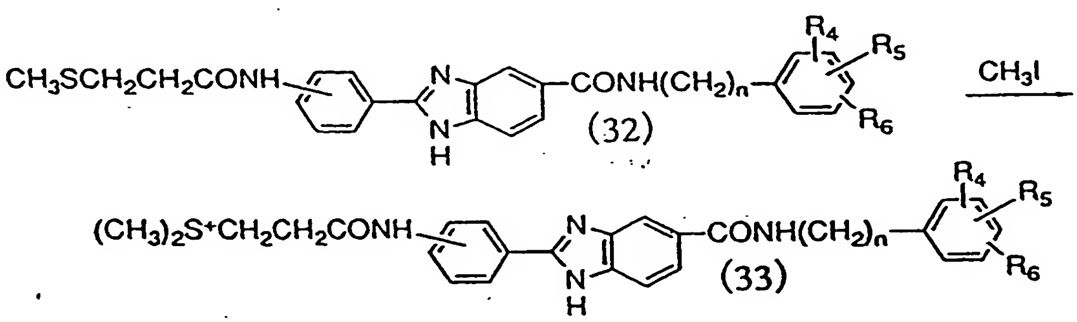
Reaction formula (12)

As a solvent for this reaction, a usual solvent is usable, but DMF or a mixed solvent containing DMF is preferred. The reaction is preferably carried out at a temperature of from 0 to 40°C for a period of from 30 minutes to 40 hours.

The employment of the following process permits the synthesis of a compound in the group B in which R_1 is a hydrogen atom, a halogen atom or a group other than the groups represented by formula (2).

For example, as shown in the following reaction formula (13), a compound represented by formula (32) can be methylated in accordance with a technique described in J. Org. Chem., Vol. 25, p. 804-807 (1960) or with the aid of a usual methylating agent (e.g., methyl iodide, dimethylsulfuric acid or methyl p-toluenesulfonate) to obtain a sulfonium derivative represented by formula (33).

Reaction formula (13):



Reaction formula (13)

As a solvent for this reaction, formic acid, acetic acid, acetone or the like can be used. Alternatively, the reaction can be carried out without solvent. The reaction is preferably carried out at a temperature of from 0 to 60°C for a period of from 1 to 60 hours.

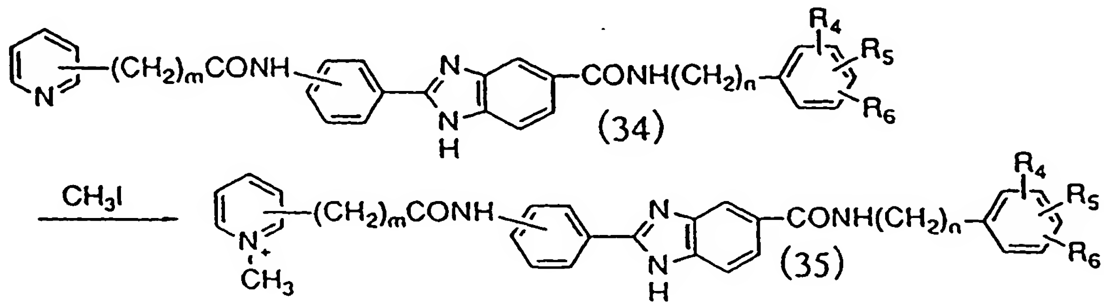
A counter anion (I^-) of the sulfonium compound obtained here can be converted into another anion in a known manner. For example, I^- can be converted into Cl^- by the use of a Cl^- type ion exchange resin (Dowex 1x8).

In the above-mentioned reaction formula (12), when a compound in which R_1 is a substituted or an unsubstituted amino group is used as the compound of formula (12), a compound in the group B can be obtained in which R_1 is the substituted or the unsubstituted amino group.

The thus obtained compound in which R_1 is the amino group substituted by two alkyl groups is further alkylated in a known manner, whereby this compound can be converted into a compound in which R_1 is an ammonium group.

Furthermore, as shown in the following reaction formula (14), a compound represented by formula (34) is methylated with methyl iodide to obtain a compound represented by formula (35).

Reaction formula (14):

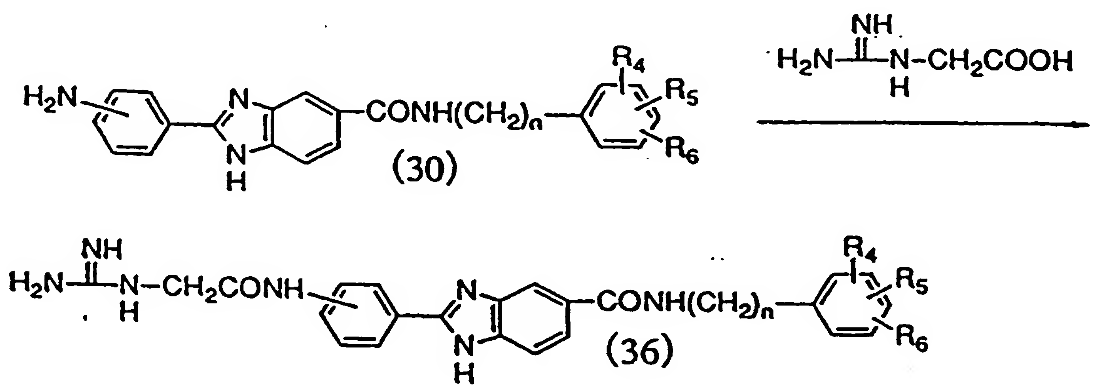


Reaction formula (14)

At this time, a usual solvent such as methanol, acetone, chloroform or methylene chloride is used. The reaction is preferably carried out at a temperature of from -5 to 50°C for a period of from 1 to 50 hours.

Moreover, as shown in the following reaction formula (15), when the amino compound of formula (30) is reacted with guanidoacetic acid, a compound of formula (36) can be synthesized in which a guanidino group is introduced into R_1.

Reaction formula (15):



Reaction formula (15)

A compound in which R_1 is an amidino group can be synthesized as follows. First, the amino compound of formula (30) is bonded to a carboxylic acid derivative having a cyano group such as 3-cyanopropionic acid in the presence of a usual condensing agent such as DCC, CDI, EDCI or DECP. As a solvent for this reaction, a usual solvent is usable, but DMF or a mixed solvent containing DMF is preferred. The reaction is preferably carried out at a temperature of from 0 to 40°C for a period of from 1 to 24 hours. The resultant product is dissolved or suspended in a solvent, and a hydrochloric acid gas is then blown thereinto, thereby obtaining an imidated compound. This compound is then dissolved or suspended in the solvent, and an ammonium gas is then blown thereinto, thereby synthesizing the desired compound in which the amidino group is introduced into R_1. As the solvent, ethanol, methanol or a mixed solvent thereof is used. In

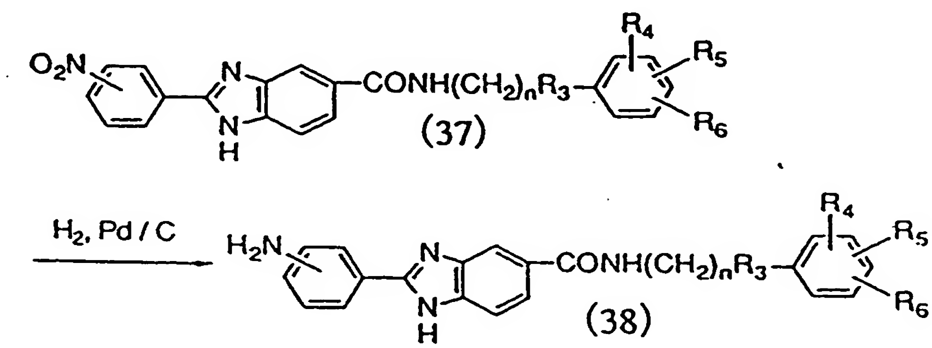
this case, the reaction is preferably carried out at a temperature of from 0 to 40°C for a period of from 30 minutes to 24 hours.

Another compound can be synthesized by changing starting materials under the above-mentioned conditions.

The compounds in the groups A and B can be synthesized as described above, but the synthesis of a compound in which both of R_1 and R_2 are groups represented by formula (2) can be accomplished, for example, as follows.

In the first place, as shown in the following reaction formula (16), a compound (a nitro compound) represented by formula (37) can be reduced to a corresponding amino group of formula (38) by catalytic hydrogenation using Pd/C as a catalyst. In this case, ethanol, methanol and DMF can be used singly or in a combination of two or more thereof as a solvent.

Reaction formula (16):

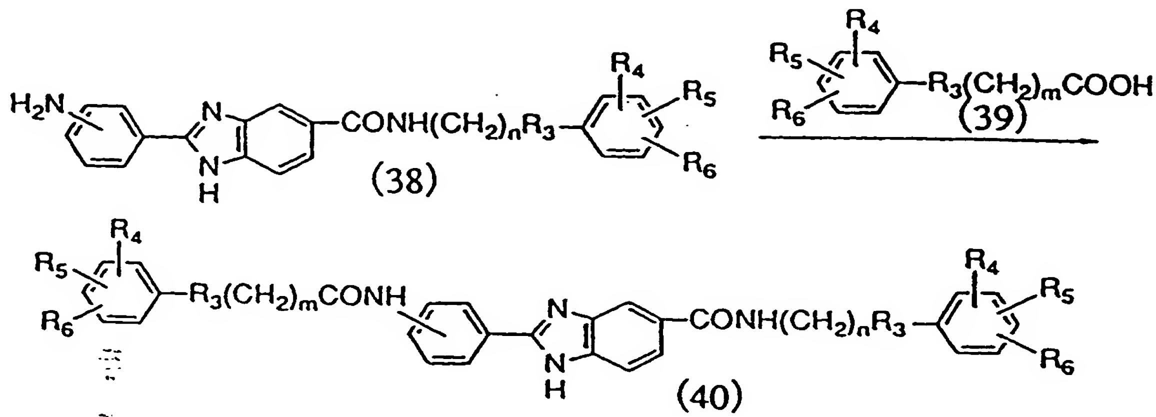


Reaction formula (16)

At this time, hydrochloric acid can be added in an amount equal to or more than mols of the nitro compound, usually in an amount of from 1 to 1.2 mols. The reaction is preferably carried out for a period of from 10 minutes to 20 hours.

Next, as shown in the following reaction formula (17), the amino compound of formula (38) can be reacted with a carboxylic acid derivative of formula (39) to obtain a compound represented by formula (40).

Reaction formula (17):



Reaction formula (17)

At this time, a usual condensing agent (e.g., DCC, CDI, EDCI or DECP) can be used. As a solvent for the above-mentioned reaction, a usual solvent is usable, but DMF or a mixed solvent containing DMF is preferred.

The reaction is preferably carried out at a temperature of from 0 to 40°C for a period of from 30 minutes to 40 hours.

Examples of the compound of formula (1) according to the present invention will be enumerated in Table 1 given below. In Table 1, a counter anion is neither shown for an ammonium group nor for a sulfonium group, but any counter anion is usable, so far as it is pharmacologically acceptable. When the counter anion is required to be specified, its typical example will be mentioned.

The compounds of the present invention can be used as anticancer agents having excellent activity. Examples of carcinomas to which the compounds of the present invention can be applied include leukemia, osteosarcoma, breast cancer, ovarian cancer, stomach cancer, colon cancer, lung cancer, and head and neck cancer. In addition, the compounds of the present invention can also be utilized as antimicrobial agents and antiviral agents.

Pharmaceutical compositions can be prepared in accordance with well-known techniques.

Various dosage forms can be selected in compliance with the purpose of a medical treatment, and typical examples of the dosage forms include solid preparations, liquid preparations and other preparations such as suppositories. These dosage forms will be described in more detail.

Examples of the solid preparations include tablets, pills, powders, granules and capsules; examples of the liquid preparations include injectable solutions, suspensions, syrups and emulsions; and examples of the other preparations include suppositories.

In preparing the pharmaceutical compositions in the form of the tablets, there can be used a wide variety of carriers which have been heretofore well known in this field. Examples of the carriers include excipients such as lactose, sucrose, sodium chloride, glucose, starch, calcium carbonate, kaolin, crystalline cellulose and silicic acid; binders such as water, ethanol, propanol, simple syrup, glucose solution, starch solution, gelatin solution, shellac solution, methyl cellulose solution, hydroxypropyl cellulose solution, polyvinyl pyrrolidone solution and carboxymethyl cellulose solution; disintegrators such as dry starch, sodium alginate, agar powder, sodium hydrogencarbonate, calcium carbonate, polyoxyethylene sorbitan fatty acid esters, sodium lauryl sulfate, stearic acid monoglyceride, starch and lactose; disintegration inhibitors such as sucrose, stearic acid, cacao butter and hydrogenated oil; absorption promoters such as quaternary ammonium bases and sodium lauryl sulfate; humectants such as glycerin and starch; adsorbents such as starch, lactose, kaolin, bentonite, colloidal silicic acid, crystalline cellulose and light anhydrous silicic acid; and lubricants such as talc, stearates, boric acid powder and polyethylene glycol.

If necessary, the tablets can also take the usual forms of coated tablets such as sugar-coated tablets, gelatin-coated tablets, enteric coated tablets, film-coated tablets, bilayer tablets or multilayer tablets.

In preparing the pharmaceutical compositions in the form of the pills, there can be used a wide variety of carriers which have been heretofore well known in this field. Examples of the carriers include excipients such as glucose, lactose,

starch, cacao butter, hardened vegetable oil, kaolin and talc; binders such as powdered acacia, powdered tragacanth and gelatin; and disintegrators such as carboxymethylcellulose calcium and agar.

The capsules can be prepared in accordance with a usual procedure, i.e., by mixing a compound as an active ingredient with any of the previously enumerated carriers, and then filling the resulting mixture into hard gelatin capsules, soft capsules or the like.

In preparing the pharmaceutical compositions in the form of the injections, solutions, emulsions or suspensions diluents are used. Examples of the diluents which can often be used in this field include water, ethanol, macrogol, propylene glycol, ethoxylated isostearyl alcohol, polyoxylated isostearyl alcohol, polyoxyethylene sorbitan fatty acid esters, cotton seed oil, corn oil, peanut oil and olive oil. Moreover, the compounds of the present invention can also be used in the form of aqueous suspensions prepared by adding water thereto in the presence of a suitable surfactant, or in the form of emulsions prepared with the aid of a surfactant such as polyoxyethylene hardened castor oil (HCO-60). Furthermore, sodium chloride, glucose and/or glycerol may be contained in the pharmaceutical compositions, and a usual solubilizer, buffering agent and/or smoothing agent may be added thereto.

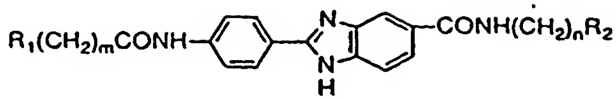
In preparing the pharmaceutical compositions in the form of suppositories, there can be used a wide variety of carriers which have been heretofore well known in this field. Examples of the carriers include polyethylene glycol, cacao butter, higher alcohols, esters of higher alcohols, gelatin and semisynthetic glycerides.

If necessary, the pharmaceutical compositions can contain colorants, preservatives, perfumes, flavors, sweeteners and/or other drugs.

No particular restriction is put on an administration manner of the pharmaceutical compositions of the present invention, and they may be administered in accordance with the form of the pharmaceutical composition, the age, sex and other conditions of a patient and the severity of a disease. For example, the tablets, pills, solutions, suspensions, emulsions, powders, granules, syrups and capsules can be orally administered. The injections can be intravenously administered alone or after being mixed with a usual infusion fluid such as glucose or an amino acid. Alternatively, they may also be administered intramuscularly, subcutaneously or intraperitoneally as required. The suppositories can be administered intrarectally. The dose of the pharmaceutical compositions of the present invention can be suitably determined in compliance with the administration manner, the age, sex and other conditions of a patient, and the severity of a disease. However, the dose should usually be determined so that the amount of the compound as the active ingredient may be in the range of from about 0.001 to 1,000 mg per day for an adult. Moreover, it is desirable that each unit dosage form of the pharmaceutical composition to be administered contains the compound as the active ingredient in an amount in the range of about 0.001 to 1,000 mg.

Generally speaking, anticancer agents, for example, even agents such as adriamycin and cisplatin which have often been used, have no small side effect. At a present technical level, the side effect should be judged in consideration of relations with functional strength, and the problem of the side effect is unavoidable to some extent. The side effect of the compounds according to the present invention is at such a level as to be acceptable as the anticancer agents.

Table 1 (1)



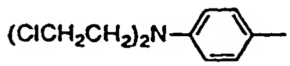
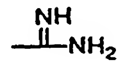
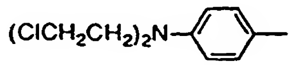
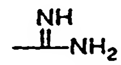
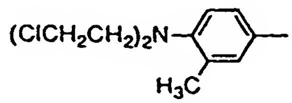
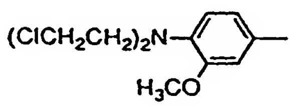
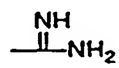
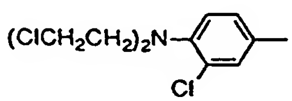
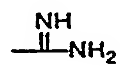
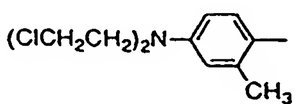
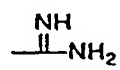
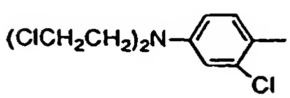
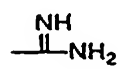
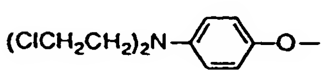
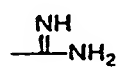
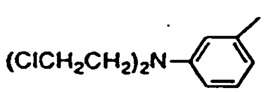
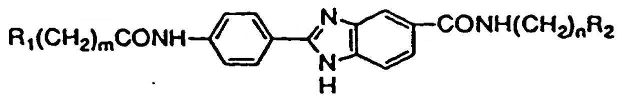
Compound No.	R_1	m	R_2	n
1		0		2
2		3		2
3		0		2
4		0		2
5		0		2
6		0		2
7		0		2
8		1		2
9		0		2

Table 1 (2)



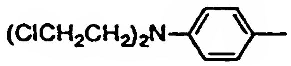
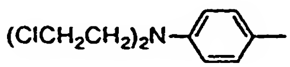
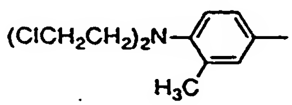
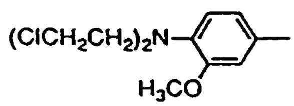
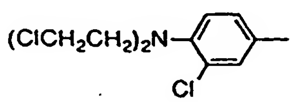
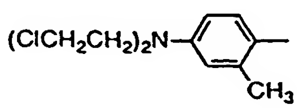
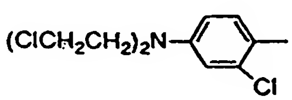
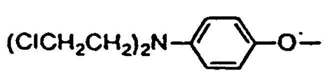
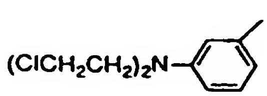
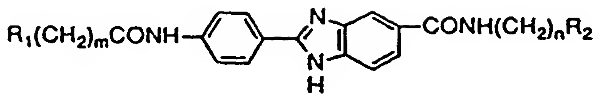
Compound No.	R_1	m	R_2	n
10		0	$-S^{+}(CH_3)-CH_3$	2
11		3	$-S^{+}(CH_3)-CH_3$	2
12		0	$-S^{+}(CH_3)-CH_3$	2
13		0	$-S^{+}(CH_3)-CH_3$	2
14		0	$-S^{+}(CH_3)-CH_3$	2
15		0	$-S^{+}(CH_3)-CH_3$	2
16		0	$-S^{+}(CH_3)-CH_3$	2
17		1	$-S^{+}(CH_3)-CH_3$	2
18		0	$-S^{+}(CH_3)-CH_3$	2

Table 1 (3)



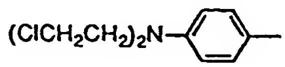
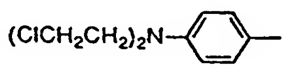
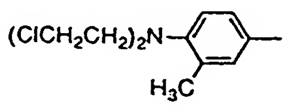
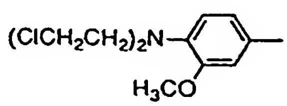
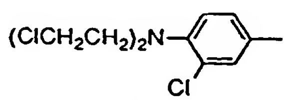
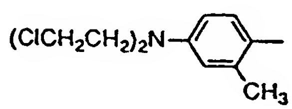
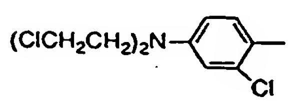
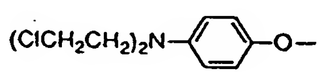
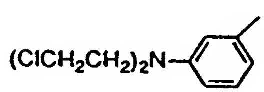
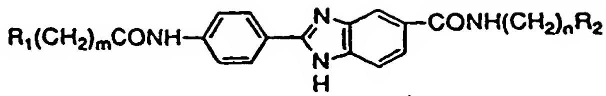
Compound No.	R_1	m	R_2	n
19		0	$-NH-C(=NH)-NH_2$	1
20		3	$-NH-C(=NH)-NH_2$	1
21		0	$-NH-C(=NH)-NH_2$	1
22		0	$-NH-C(=NH)-NH_2$	1
23		0	$-NH-C(=NH)-NH_2$	1
24		0	$-NH-C(=NH)-NH_2$	1
25		0	$-NH-C(=NH)-NH_2$	1
26		1	$-NH-C(=NH)-NH_2$	1
27		0	$-NH-C(=NH)-NH_2$	1

Table 1 (4)



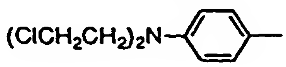
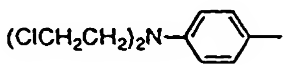
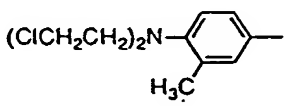
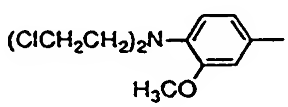
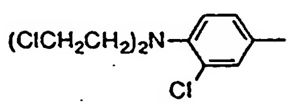
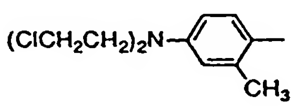
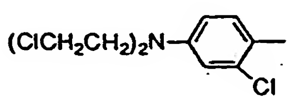
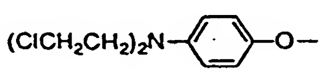
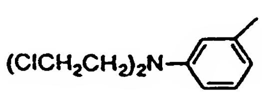
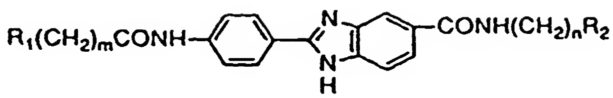
Compound No.	R_1	m	R_2	n
28		0	$-N(CH_3)-CH_3$	3
29		3	$-N(CH_3)-CH_3$	3
30		0	$-N(CH_3)-CH_3$	3
31		0	$-N(CH_3)-CH_3$	3
32		0	$-N(CH_3)-CH_3$	3
33		0	$-N(CH_3)-CH_3$	3
34		0	$-N(CH_3)-CH_3$	3
35		1	$-N(CH_3)-CH_3$	3
36		0	$-N(CH_3)-CH_3$	3

Table 1 (5)



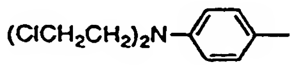
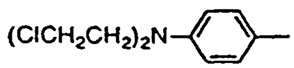
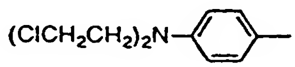
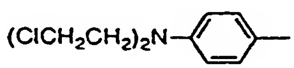
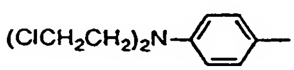
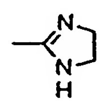
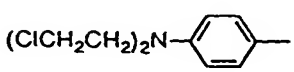
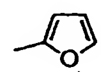
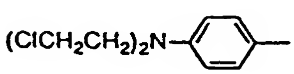
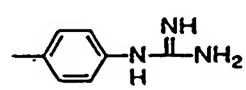
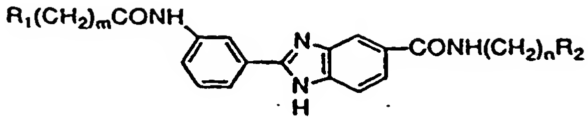
Compound No.	R_1	m	R_2	n
37		0	$-Cl$	2
38		0	$-Br$	2
39		0	$-S-CH_3$	2
40		0	$-N^+(CH_3)_3$	3
41		0		3
42		0		0
43		0		0
44	H	0	$-N(CH_3)-CH_3$	3
45	H	0	$-C(=NH)-NH_2$	2

Table 1 (6)



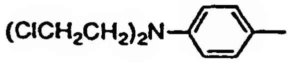
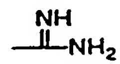
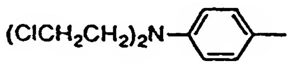
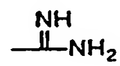
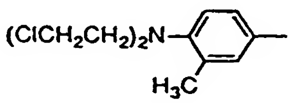
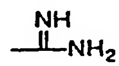
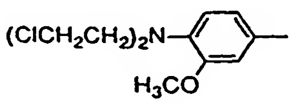
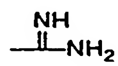
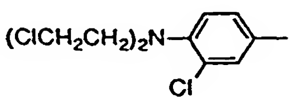
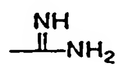
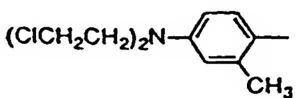
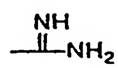
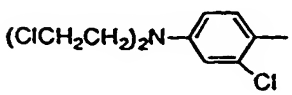
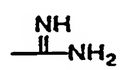
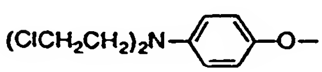
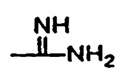
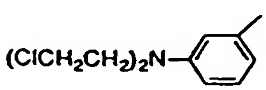
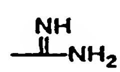
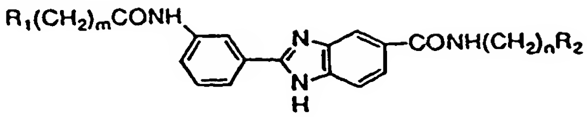
Compound No.	R_1	m	R_2	n
46		0		2
47		3		2
48		0		2
49		0		2
50		0		2
51		0		2
52		0		2
53		1		2
54		0		2

Table 1 (7)



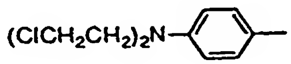
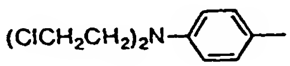
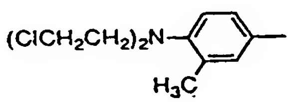
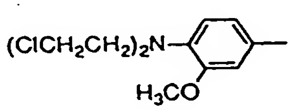
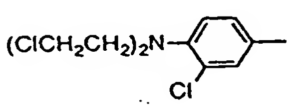
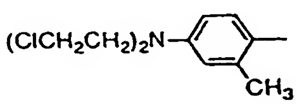
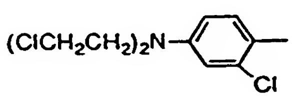
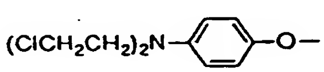
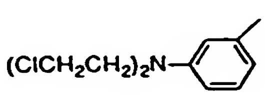
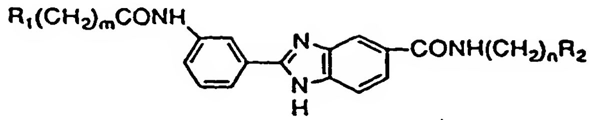
Compound No.	R_1	m	R_2	n
55		0	$-S^{+}(CH_3)-CH_3$	2
56		3	$-S^{+}(CH_3)-CH_3$	2
57		0	$-S^{+}(CH_3)-CH_3$	2
58		0	$-S^{+}(CH_3)-CH_3$	2
59		0	$-S^{+}(CH_3)-CH_3$	2
60		0	$-S^{+}(CH_3)-CH_3$	2
61		0	$-S^{+}(CH_3)-CH_3$	2
62		1	$-S^{+}(CH_3)-CH_3$	2
63		0	$-S^{+}(CH_3)-CH_3$	2

Table 1 (8)



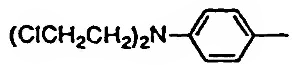
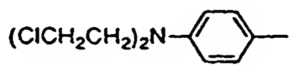
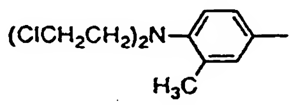
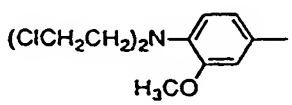
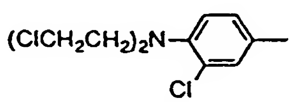
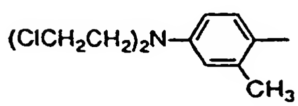
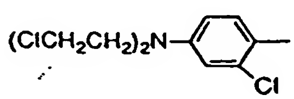
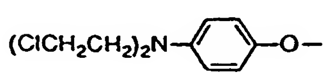
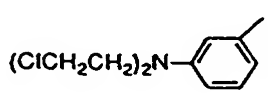
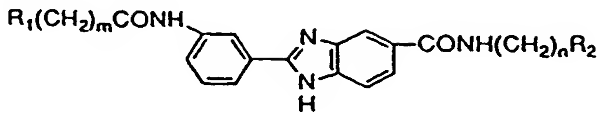
Compound No.	R_1	m	R_2	n
64		0	$-NH-C(=NH)-NH_2$	1
65		3	$-NH-C(=NH)-NH_2$	1
66		0	$-NH-C(=NH)-NH_2$	1
67		0	$-NH-C(=NH)-NH_2$	1
68		0	$-NH-C(=NH)-NH_2$	1
69		0	$-NH-C(=NH)-NH_2$	1
70		0	$-NH-C(=NH)-NH_2$	1
71		1	$-NH-C(=NH)-NH_2$	1
72		0	$-NH-C(=NH)-NH_2$	1

Table 1 (9)



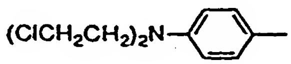
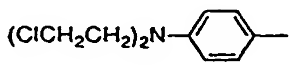
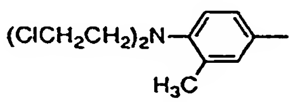
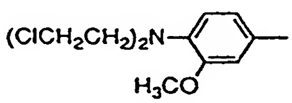
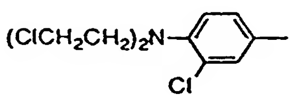
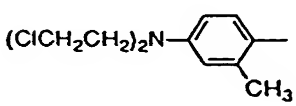
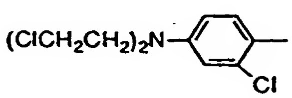
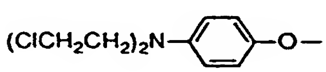
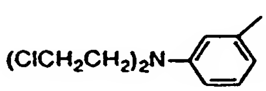
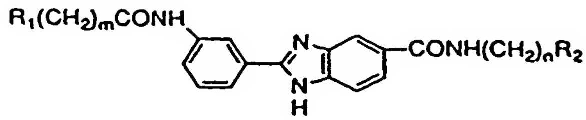
Compound No.	R_1	m	R_2	n
73		0	$-N(CH_3)-CH_3$	3
74		3	$-N(CH_3)-CH_3$	3
75		0	$-N(CH_3)-CH_3$	3
76		0	$-N(CH_3)-CH_3$	3
77		0	$-N(CH_3)-CH_3$	3
78		0	$-N(CH_3)-CH_3$	3
79		0	$-N(CH_3)-CH_3$	3
80		1	$-N(CH_3)-CH_3$	3
81		0	$-N(CH_3)-CH_3$	3

Table 1 (10)



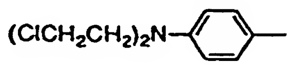
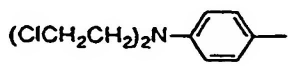
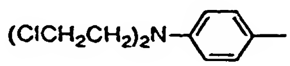
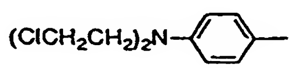
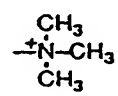
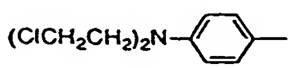
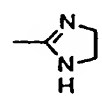
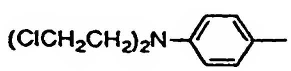
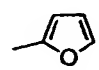
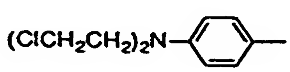
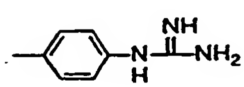
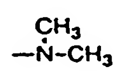
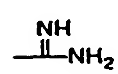
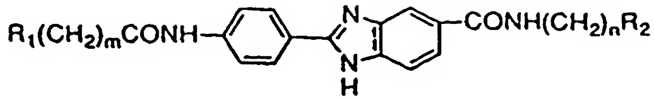
Compound No.	R_1	m	R_2	n
82		0	$-Cl$	2
83		0	$-Br$	2
84		0	$-S-CH_3$	2
85		0		3
86		0		3
87		0		0
88		0		0
89	H	0		3
90	H	0		2

Table 1 (11)



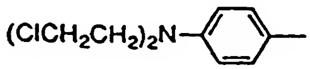
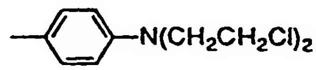
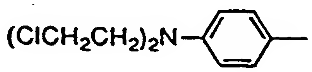
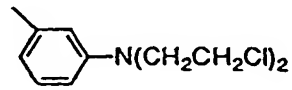
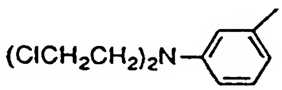
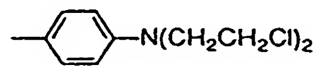
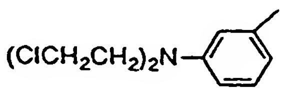
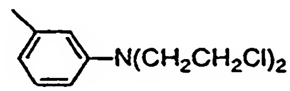
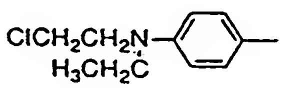
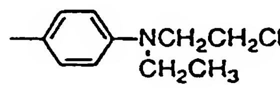
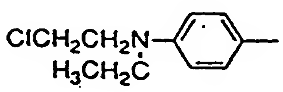
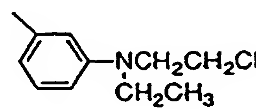
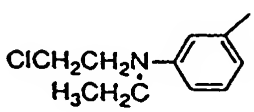
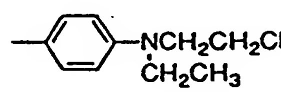
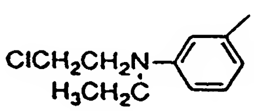
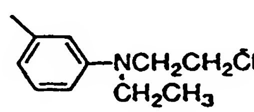
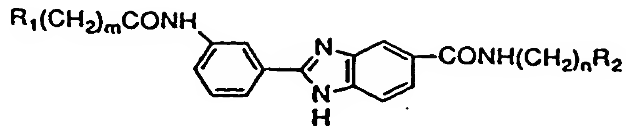
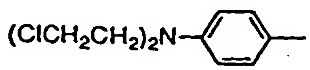
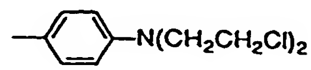
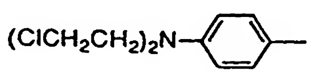
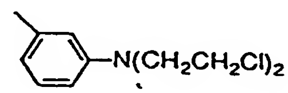
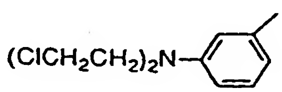
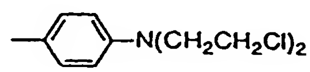
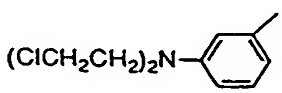
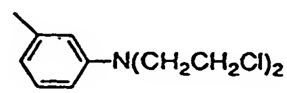
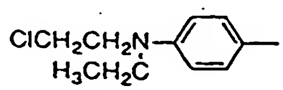
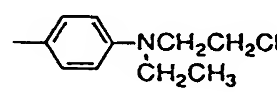
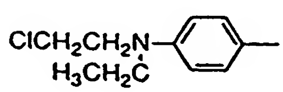
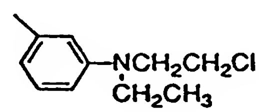
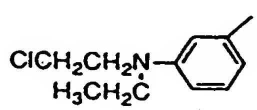
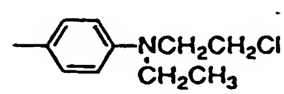
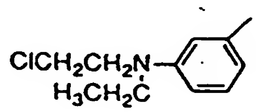
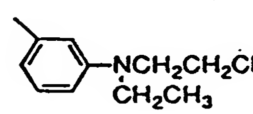
Compound No.	R_1	m	R_2	n
91		0		0
92		0		0
93		0		0
94		0		0
95		0		0
96		0		0
97		0		0
98		0		0

Table 1 (12)



Compound No.	R_1	m	R_2	n
99		0		0
100		0		0
101		0		0
102		0		0
103		0		0
104		0		0
105		0		0
106		0		0

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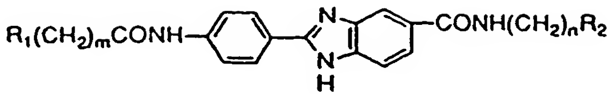

Compound No.	R_1	m	R_2	n
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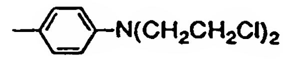
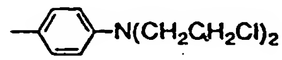
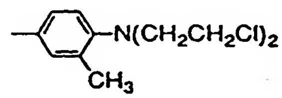
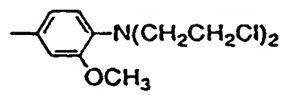
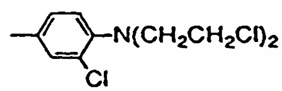
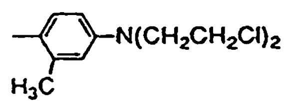
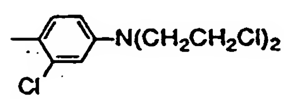
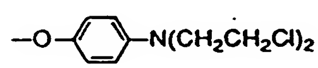
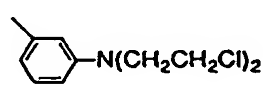
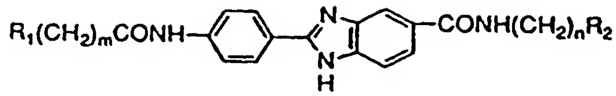
1002	$H_2N-C(=NH)-NH-$	1		2
1003	$H_2N-C(=NH)-NH-$	1		3
1004	$H_2N-C(=NH)-NH-$	1		0
1005	$H_2N-C(=NH)-NH-$	1		0
1006	$H_2N-C(=NH)-NH-$	1		0
1007	$H_2N-C(=NH)-NH-$	1		0
1008	$H_2N-C(=NH)-NH-$	1		0
1009	$H_2N-C(=NH)-NH-$	1		1
1010	$H_2N-C(=NH)-NH-$	1		0

Table 1 (14)



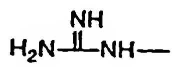
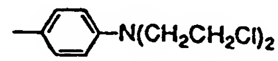
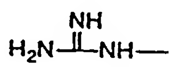
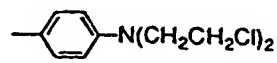
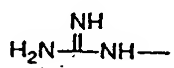
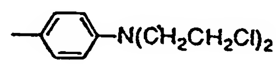
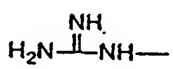
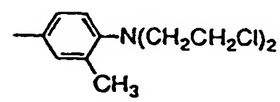
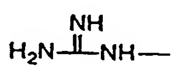
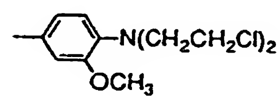
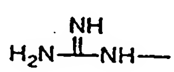
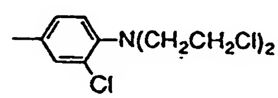
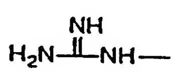
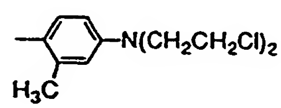
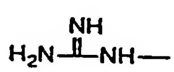
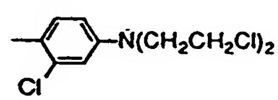
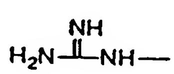
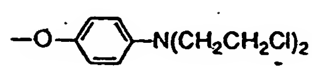
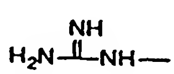
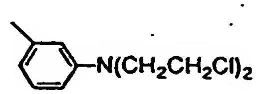
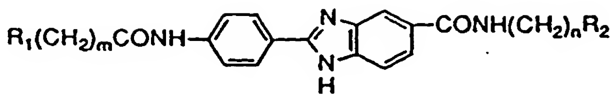
Compound No.	R_1	m	R_2	n
1011		3		0
1012		3		2
1013		3		3
1014		3		0
1015		3		0
1016		3		0
1017		3		0
1018		3		0
1019		3		1
1020		3		0

Table 1 (15)



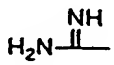
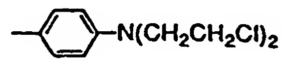
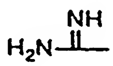
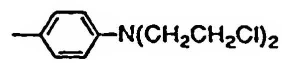
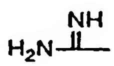
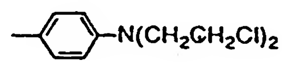
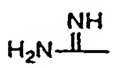
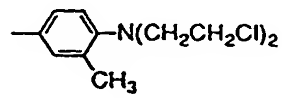
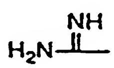
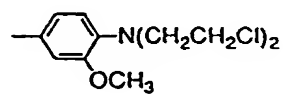
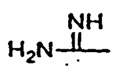
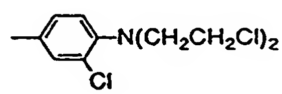
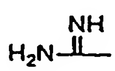
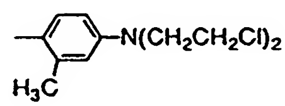
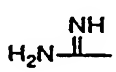
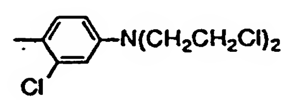
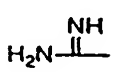
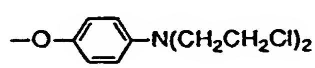
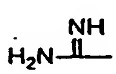
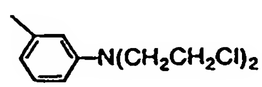
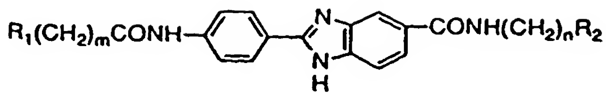
Compound No.	R_1	m	R_2	n
1021		3		0
1022		3		2
1023		3		3
1024		3		0
1025		3		0
1026		3		0
1027		3		0
1028		3		0
1029		3		1
1030		3		0

Table 1 (16)



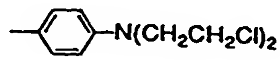
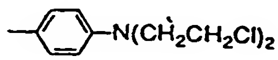
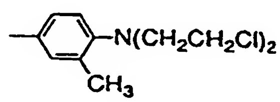
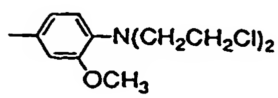
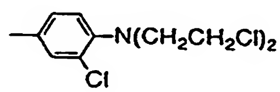
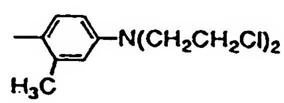
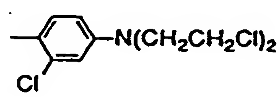
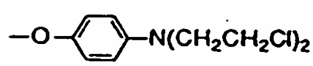
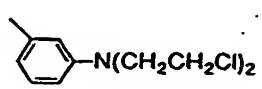
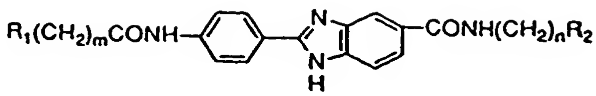
Compound No.	R_1	m	R_2	n
1031	$H_3C-N(CH_3)-$	3		0
1032	$H_3C-N(CH_3)-$	3		2
1033	$H_3C-N(CH_3)-$	3		3
1034	$H_3C-N(CH_3)-$	3		0
1035	$H_3C-N(CH_3)-$	3		0
1036	$H_3C-N(CH_3)-$	3		0
1037	$H_3C-N(CH_3)-$	3		0
1038	$H_3C-N(CH_3)-$	3		0
1039	$H_3C-N(CH_3)-$	3		1
1040	$H_3C-N(CH_3)-$	3		0

Table 1 (17)



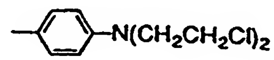
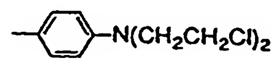
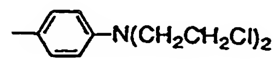
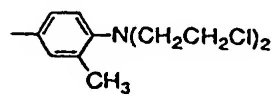
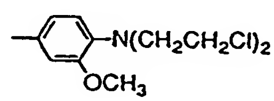
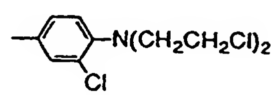
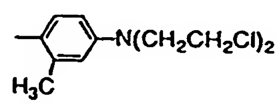
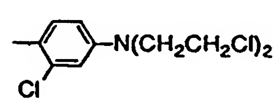
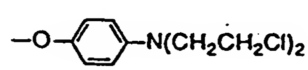
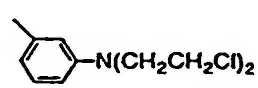
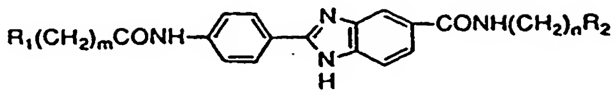
Compound No.	R_1	m	R_2	n
1041	$H_3C-\overset{CH_3}{\dot{S}}{}^{+}-$	2		0
1042	$H_3C-\overset{CH_3}{\dot{S}}{}^{+}-$	2		2
1043	$H_3C-\overset{CH_3}{\dot{S}}{}^{+}-$	2		3
1044	$H_3C-\overset{CH_3}{\dot{S}}{}^{+}-$	2		0
1045	$H_3C-\overset{CH_3}{\dot{S}}{}^{+}-$	2		0
1046	$H_3C-\overset{CH_3}{\dot{S}}{}^{+}-$	2		0
1047	$H_3C-\overset{CH_3}{\dot{S}}{}^{+}-$	2		0
1048	$H_3C-\overset{CH_3}{\dot{S}}{}^{+}-$	2		0
1049	$H_3C-\overset{CH_3}{\dot{S}}{}^{+}-$	2		1
1050	$H_3C-\overset{CH_3}{\dot{S}}{}^{+}-$	2		0

Table 1 (18)



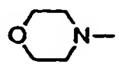
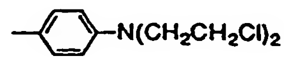
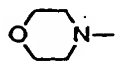
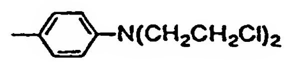
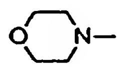
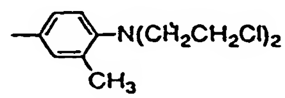
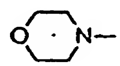
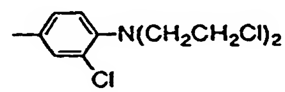
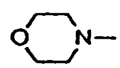
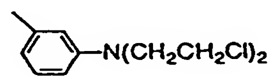
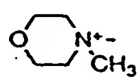
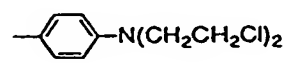
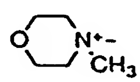
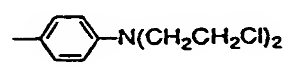
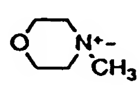
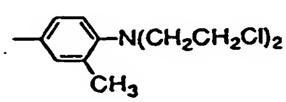
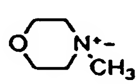
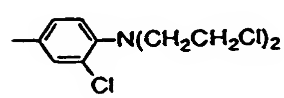
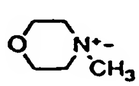
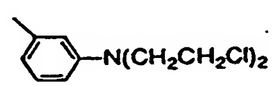
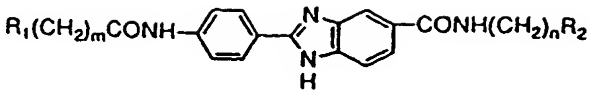
Compound No.	R_1	m	R_2	n
1051		2		0
1052		2		3
1053		2		0
1054		2		0
1055		2		0
1056		2		0
1057		2		3
1058		2		0
1059		2		0
1060		2		0

Table 1 (19)



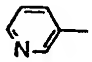
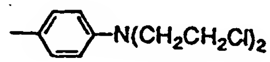
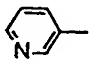
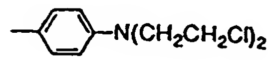
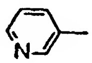
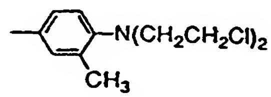
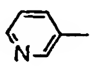
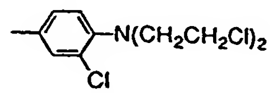
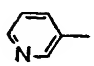
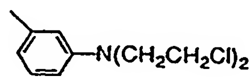
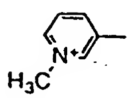
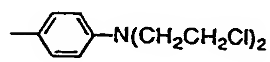
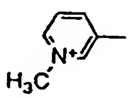
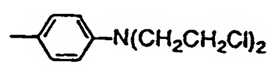
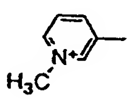
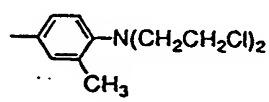
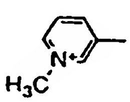
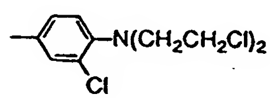
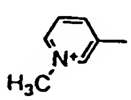
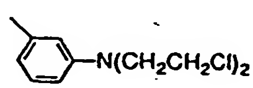
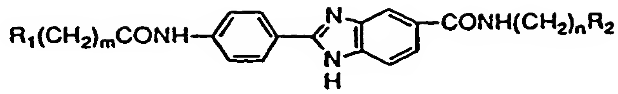
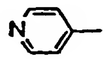
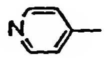
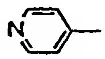
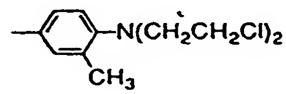
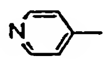
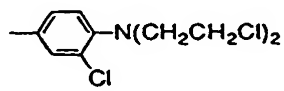
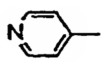
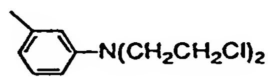
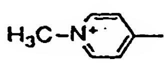
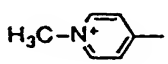
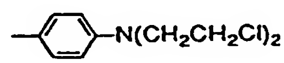
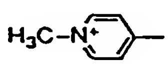
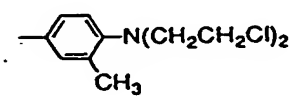
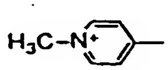
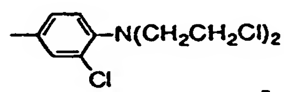
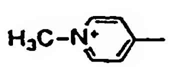
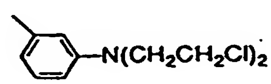
Compound No.	R_1	m	R_2	n
1061		1		0
1062		1		3
1063		1		0
1064		1		0
1065		1		0
1066		1		0
1067		1		3
1068		1		0
1069		1		0
1070		1		0

Table 1 (20)



Compound No.	R_1	m	R_2	n
1071		1		0
1072		1		3
1073		1		0
1074		1		0
1075		1		0
1076		1		0
1077		1		3
1078		1		0
1079		1		0
1080		1		0

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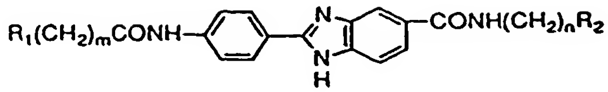

Compound No.	R_1	m	R_2	n
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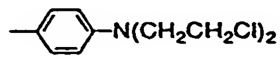
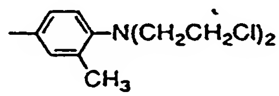
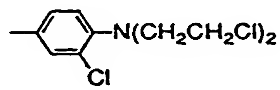
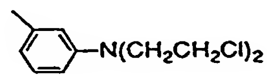
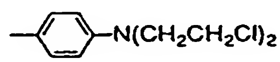
1082	$H_3C-S^+(CH_3)-$	1		3
1083	$H_3C-S^+(CH_3)-$	1		0
1084	$H_3C-S^+(CH_3)-$	1		0
1085	$H_3C-S^+(CH_3)-$	1		0
1086	$H_3C-S^+(CH_3)-$	3		0
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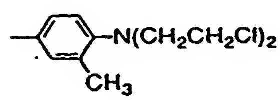
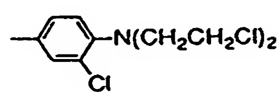
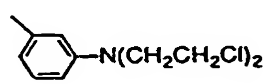
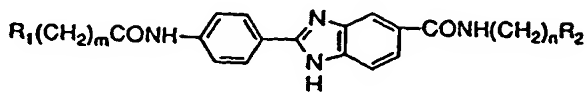
1088	$H_3C-S^+(CH_3)-$	3		0
1089	$H_3C-S^+(CH_3)-$	3		0
1090	$H_3C-S^+(CH_3)-$	3		0

Table 1 (22)



Compound No.	R_1	m	R_2	n
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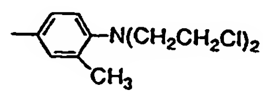
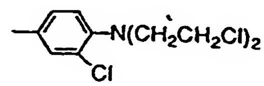
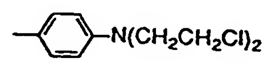
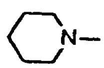
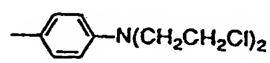
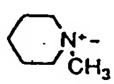
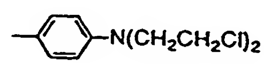
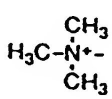
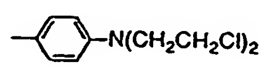
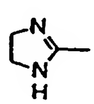
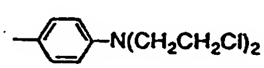
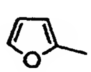
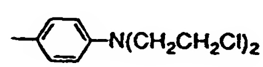
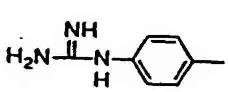
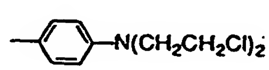
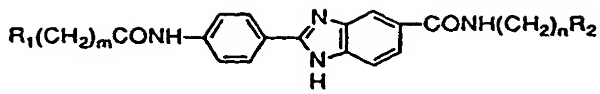
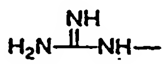
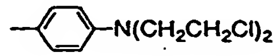
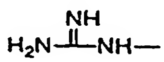
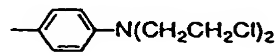
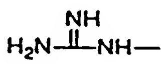
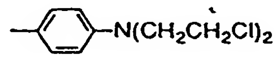
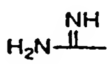
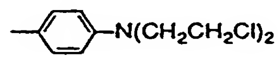
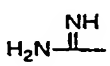
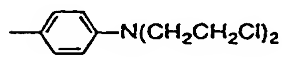
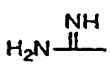
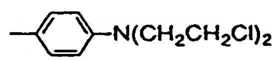
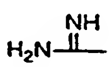
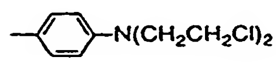
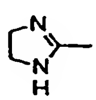
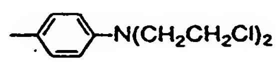
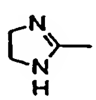
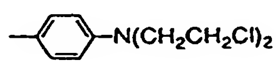
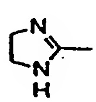
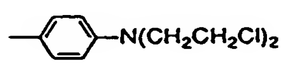
1092	H_3C-S-	2		0
1093	H_3C-S-	2		0
1094	$Cl-$	2		0
1095		2		0
1096		2		0
1097		3		0
1098		3		0
1099		0		0
1100		0		0

Table 1 (23)



Compound No.	R_1	m	R_2	n
1101		2		0
1102		4		0
1103		5		0
1104		1		0
1105		2		0
1106		4		0
1107		5		0
1108		1		0
1109		2		0
1110		3		0

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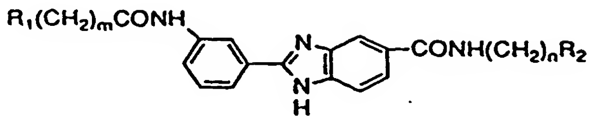
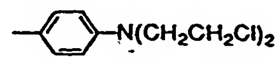
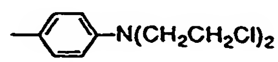
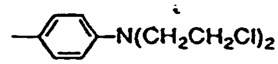
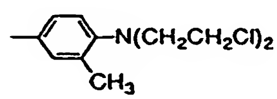
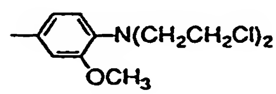
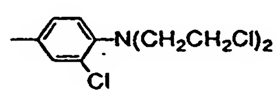
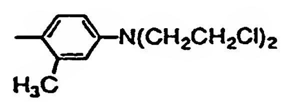
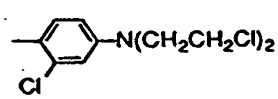
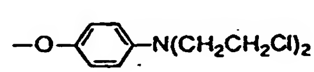
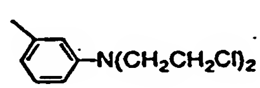


Compound No.	R_1	m	R_2	n
2001	$H_2N-C(=NH)-NH-$	1		0
2002	$H_2N-C(=NH)-NH-$	1		2
2003	$H_2N-C(=NH)-NH-$	1		3
2004	$H_2N-C(=NH)-NH-$	1		0
2005	$H_2N-C(=NH)-NH-$	1		0
2006	$H_2N-C(=NH)-NH-$	1		0
2007	$H_2N-C(=NH)-NH-$	1		0
2008	$H_2N-C(=NH)-NH-$	1		0
2009	$H_2N-C(=NH)-NH-$	1		1
2010	$H_2N-C(=NH)-NH-$	1		0

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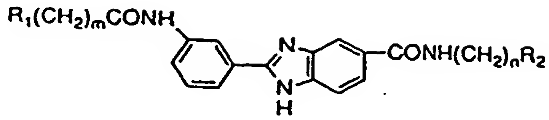
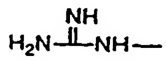
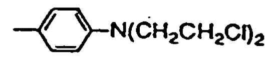
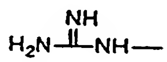
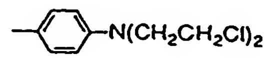
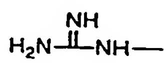
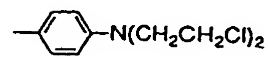
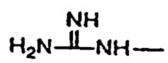
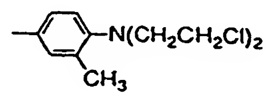
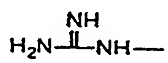
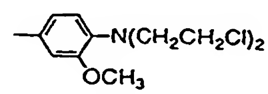
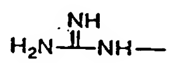
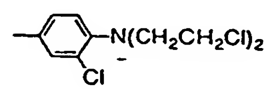
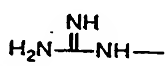
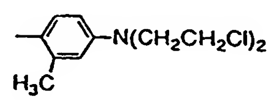
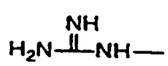
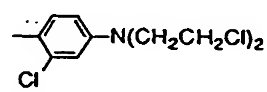
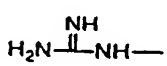
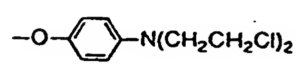
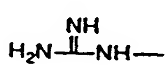
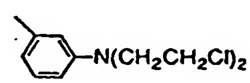


Compound No.	R_1	m	R_2	n
2011		3		0
2012		3		2
2013		3		3
2014		3		0
2015		3		0
2016		3		0
2017		3		0
2018		3		0
2019		3		1
2020		3		0

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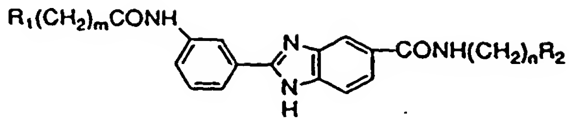
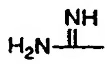
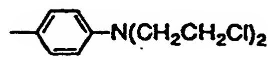
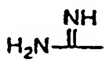
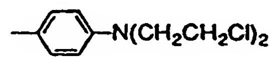
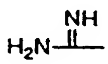
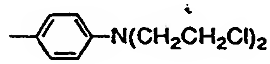
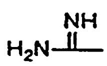
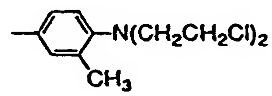
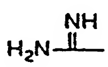
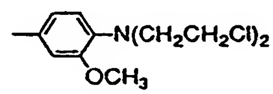
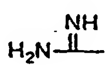
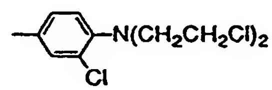
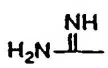
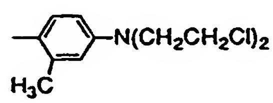
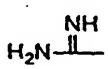
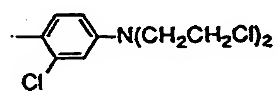
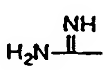
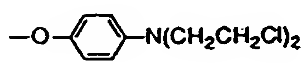
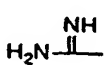
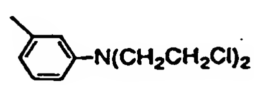


Compound No.	R_1	m	R_2	n
2021		3		0
2022		3		2
2023		3		3
2024		3		0
2025		3		0
2026		3		0
2027		3		0
2028		3		0
2029		3		1
2030		3		0

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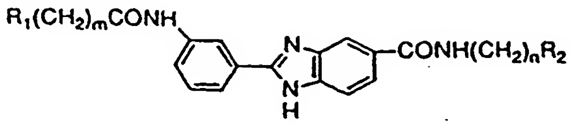


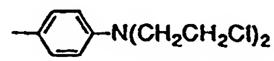
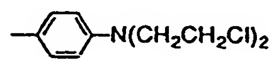
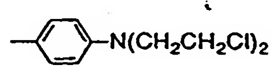
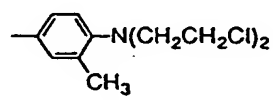
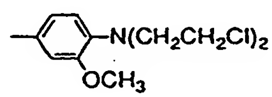
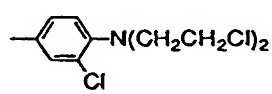
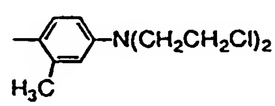
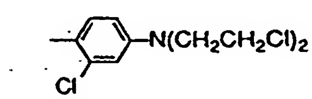
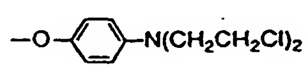
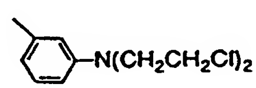
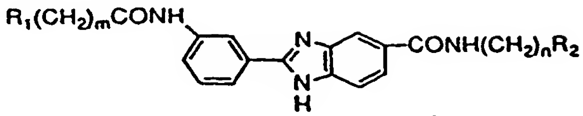
Compound No.	R_1	m	R_2	n
2031	$H_3C-\overset{CH_3}{N}-$	3		0
2032	$H_3C-\overset{CH_3}{N}-$	3		2
2033	$H_3C-\overset{CH_3}{N}-$	3		3
2034	$H_3C-\overset{CH_3}{N}-$	3		0
2035	$H_3C-\overset{CH_3}{N}-$	3		0
2036	$H_3C-\overset{CH_3}{N}-$	3		0
2037	$H_3C-\overset{CH_3}{N}-$	3		0
2038	$H_3C-\overset{CH_3}{N}-$	3		0
2039	$H_3C-\overset{CH_3}{N}-$	3		1
2040	$H_3C-\overset{CH_3}{N}-$	3		0

Table 1 (28)



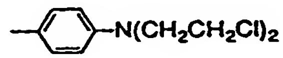
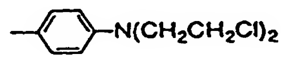
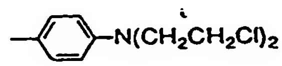
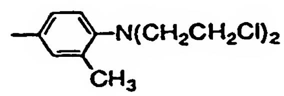
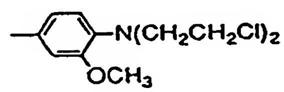
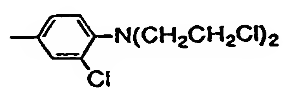
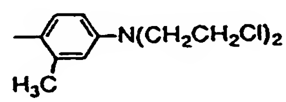
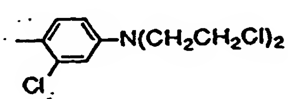
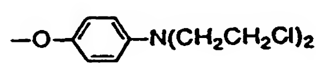
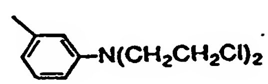
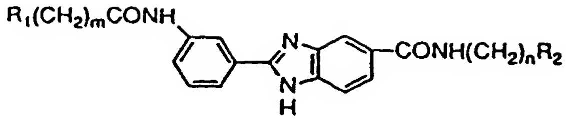
Compound No.	R_1	m	R_2	n
2041	$H_3C-\overset{CH_3}{\dot{S}^+}-$	2		0
2042	$H_3C-\overset{CH_3}{\dot{S}^+}-$	2		2
2043	$H_3C-\overset{CH_3}{\dot{S}^+}-$	2		3
2044	$H_3C-\overset{CH_3}{\dot{S}^+}-$	2		0
2045	$H_3C-\overset{CH_3}{\dot{S}^+}-$	2		0
2046	$H_3C-\overset{CH_3}{\dot{S}^+}-$	2		0
2047	$H_3C-\overset{CH_3}{\dot{S}^+}-$	2		0
2048	$H_3C-\overset{CH_3}{\dot{S}^+}-$	2		0
2049	$H_3C-\overset{CH_3}{\dot{S}^+}-$	2		1
2050	$H_3C-\overset{CH_3}{\dot{S}^+}-$	2		0

Table 1 (29)



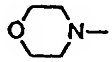
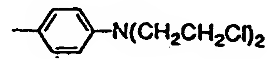
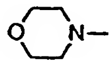
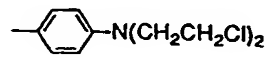
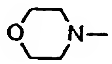
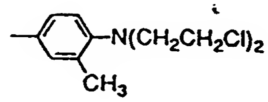
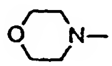
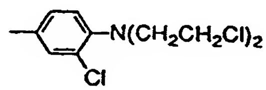
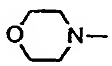
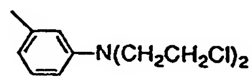
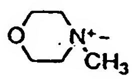
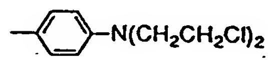
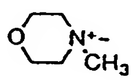
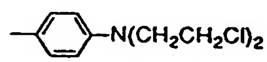
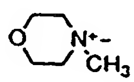
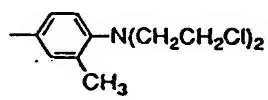
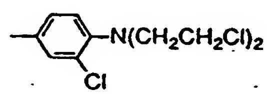
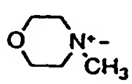
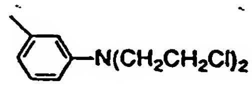
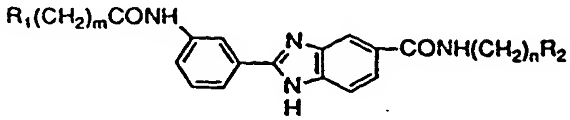
Compound No.	R_1	m	R_2	n
2051		2		0
2052		2		3
2053		2		0
2054		2		0
2055		2		0
2056		2		0
2057		2		3
2058		2		0
2059		2		0
2060		2		0

Table 1 (30)



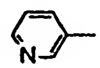
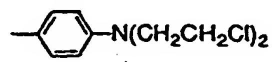
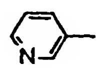
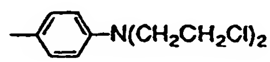
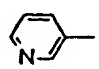
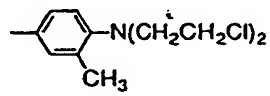
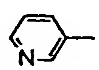
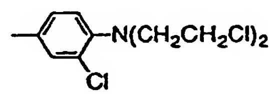
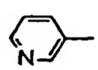
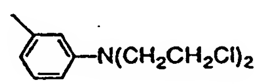
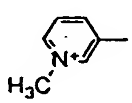
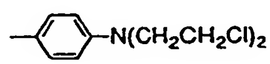
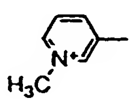
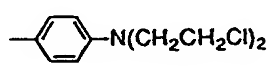
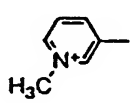
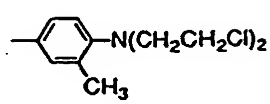
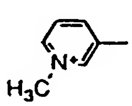
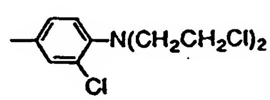
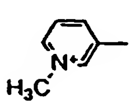
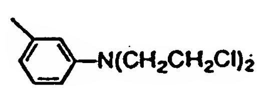
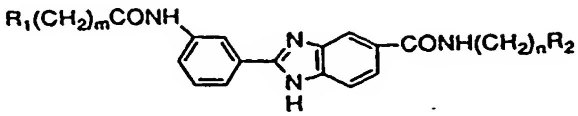
Compound No.	R_1	m	R_2	n
2061		1		0
2062		1		3
2063		1		0
2064		1		0
2065		1		0
2066		1		0
2067		1		3
2068		1		0
2069		1		0
2070		1		0

Table 1 (31)



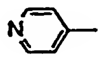
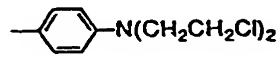
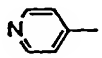
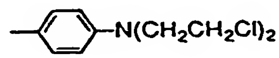
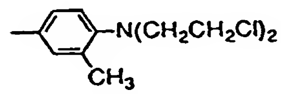
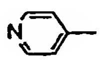
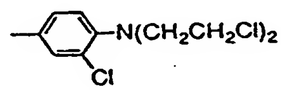
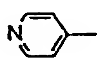
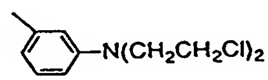
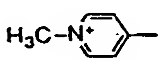
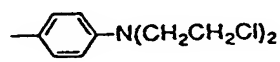
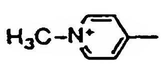
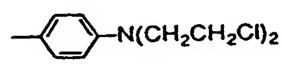
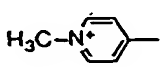
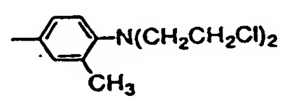
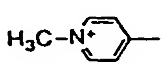
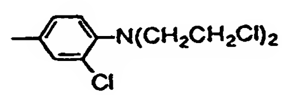
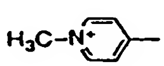
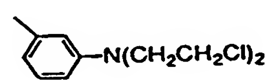
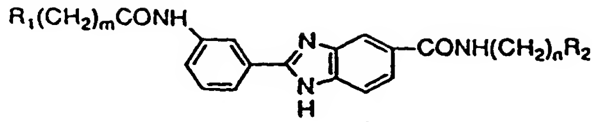
Compound No.	R_1	m	R_2	n
2071		1		0
2072		1		3
2073		1		0
2074		1		0
2075		1		0
2076		1		0
2077		1		3
2078		1		0
2079		1		0
2080		1		0

Table 1 (32)



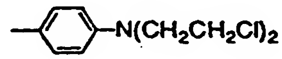
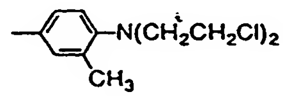
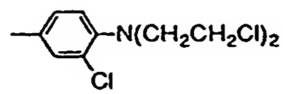
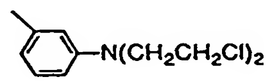
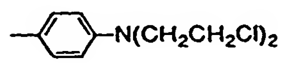
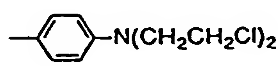
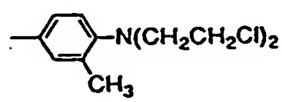
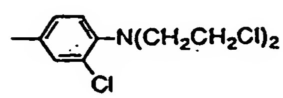
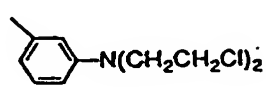
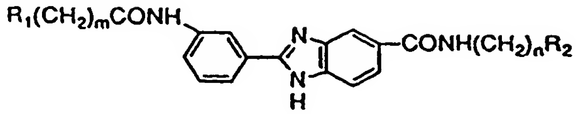
Compound No.	R_1	m	R_2	n
2081	$H_3C\text{-}\overset{CH_3}{S^+}\text{-}$	1		0
2082	$H_3C\text{-}\overset{CH_3}{S^+}\text{-}$	1		3
2083	$H_3C\text{-}\overset{CH_3}{S^+}\text{-}$	1		0
2084	$H_3C\text{-}\overset{CH_3}{S^+}\text{-}$	1		0
2085	$H_3C\text{-}\overset{CH_3}{S^+}\text{-}$	1		0
2086	$H_3C\text{-}\overset{CH_3}{S^+}\text{-}$	3		0
2087	$H_3C\text{-}\overset{CH_3}{S^+}\text{-}$	3		3
2088	$H_3C\text{-}\overset{CH_3}{S^+}\text{-}$	3		0
2089	$H_3C\text{-}\overset{CH_3}{S^+}\text{-}$	3		0
2090	$H_3C\text{-}\overset{CH_3}{S^+}\text{-}$	3		0

Table 1 (33)



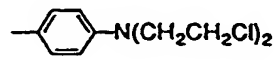
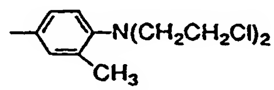
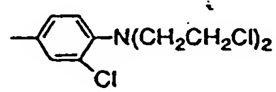
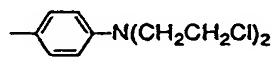
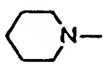
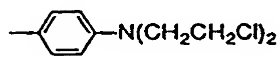
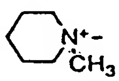
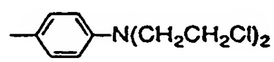
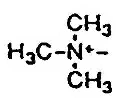
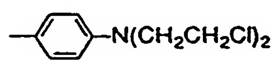
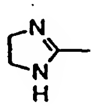
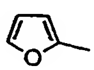
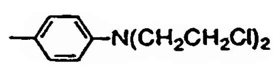
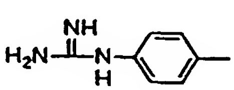
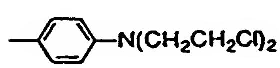
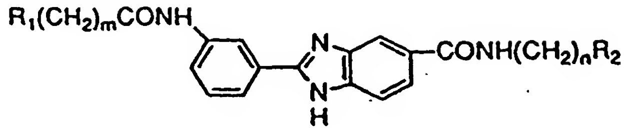
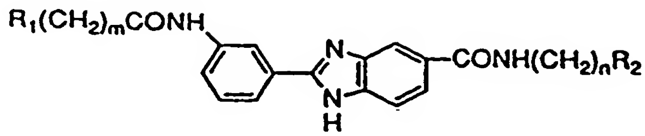
Compound No.	R_1	m	R_2	n
2091	H_3C-S—	2		0
2092	H_3C-S—	2		0
2093	H_3C-S—	2		0
2094	Cl—	2		0
2095		2		0
2096		2		0
2097		3		0
2098		3		0
2099		0		0
2100		0		0

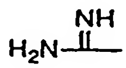
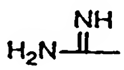
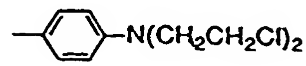
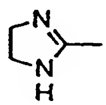
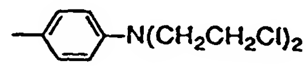
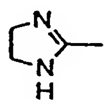
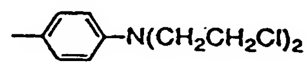
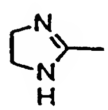
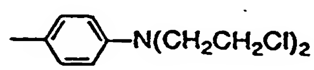
Table 1 (34)



Compound No.	R_1	m	R_2	n
2101	$H_2N-C(=NH)-NH-$	2	$-C_6H_4-N(CH_2CH_2Cl)_2$	0
2102	$H_2N-C(=NH)-NH-$	4	$-C_6H_4-N(CH_2CH_2Cl)_2$	0
2103	$H_2N-C(=NH)-NH-$	5	$-C_6H_4-N(CH_2CH_2Cl)_2$	0
2104	$H_2N-C(=NH)-$	1	$-C_6H_4-N(CH_2CH_2Cl)_2$	0
2105	$H_2N-C(=NH)-$	2	$-C_6H_4-N(CH_2CH_2Cl)_2$	0

Table 1 (35)



Compound No.	R_1	m	R_2	n
2106		4		0
2107		5		0
2108		1		0
2109		2		0
2110		3		0

The numbers of compounds in the undermentioned examples correspond to those of the compounds enumerated in Table 1.

Example 1 (Compound 47)

1H-2-[3-[[4-[4-[N,N-bis(2-chloroethyl)amino]phenyl]butyryl]amino]phenyl]benzimidazole-5-[N-(2-amidinoethyl]carboxyamide hydrochloride

Reaction 1

1H-2-(3-nitrophenyl)benzimidazole-5-carboxylic acid

In 100 ml of nitrobenzene were dissolved 2.0 g (13.1 mmols, 1.0 eq.) of 3,4-diaminobenzoic acid and 2.0 g (13.2 mmols) of m-nitrobenzaldehyde, and the mixture was then heated and stirred for 21.5 hours in an oil bath at 155°C. The resulting solid was collected by filtration, and then washed with IPA to obtain 1.88 g (6.64 mmols, 50.3%) of the desired compound in the state of yellowish green white crystals.

mp: >270°C

NMR (DMSO-d_6) δ: 9.03 (s, 1H), 8.64 (d, 1H), 8.36 (d, 1H), 8.31-8.17 (m, 1H), 7.89 (t, 2H), 7.80-7.68 (m, 1H)

Reaction 2

1H-2-(3-nitrophenyl)benzimidazole-5-[N-(2-cyanoethyl)]carboxyamide

1.0 g (3.53 mmols) of 1H-2-(3-nitrophenyl)benzimidazole-5-carboxylic acid was suspended in 20 ml of DMF, and 0.69 g (4.26 mmols, 1.2 eq.) of CDI was then added, followed by stirring at room temperature in a nitrogen atmosphere. After 3.5 hours, the solution was cooled on ice. Then, 0.27 ml (3.65 mmols, 1.0 eq.) of β-aminopropionitrile was added to the solution, and the temperature of the solution was then returned to room temperature. After stirring for 3 hours, the solution was then allowed to stand overnight. Next, the solution was concentrated under reduced pressure, and the resulting residue was crystallized from methanol, thereby obtaining 1.08 g (3.19 mmols, 90.4%) of the desired compound in the state of ocher crystals.

mp: >270°C

NMR (DMSO-d_6) δ: 9.04 (s, 1H), 8.90 (m, 1H), 8.64 (d, 1H), 8.37 (d, 1H), 8.25-8.14 (m, 1H), 7.89 (t, 1H), 7.83-7.67 (m, 2H), 3.54 (q, 2H), 2.82 (t, 2H)

Reaction 3

1H-2-(3-nitrophenyl)benzimidazole-5-[N-(2-amidinoethyl)]carboxyamide hydrochloride

1.08 g (3.22 mmols) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-(2-cyanoethyl)]carboxyamide was suspended in 40 ml of ethanol, and a hydrochloric acid gas was then blown into the suspension under ice cooling. After the suspension was saturated with a hydrochloric acid gas over 30 minutes, the temperature of the suspension was returned to room temperature, followed by stirring for 3 hours. Next, the suspension was concentrated under reduced pressure. The resulting residue was decanted twice with ether and then suspended in 40 ml of ethanol, and an ammonia gas was blown thereinto under ice cooling. After saturated with the ammonia gas over 50 minutes, the suspension was stirred at room temperature for 3 hours, and then allowed to stand overnight. Next, the suspension was concentrated under reduced pressure, and the resulting residue was then sludged with methanol/acetone. The resulting solid was purified through silica gel column chromatography (ethyl acetate/IPA/water = 5/2/1), and then sludged with methylene chloride and successively IPA to obtain 0.67 g (1.72 mmols, 53.5%) of the desired compound in the state of creamy crystals.

mp: >270°C

NMR (DMSO-d_6) δ: 9.10-9.06 (m, 3H), 8.88 (t, 0.5H), 8.79 (t, 0.5H), 8.71 (bs, 3H), 8.35 (d, 1H), 8.31 (s, 0.5H), 8.15 (s, 0.5H), 7.88 (t, 1H), 7.88 (d, 1H), 7.75 (d, 0.5H), 7.63 (d, 1H), 3.65 (q, 2H), 2.73 (t, 2H)

Reaction 4

1H-2-[3-[[4-[4-[N,N-bis(2-chloroethyl)amino]phenyl]butyryl]amino]phenyl]benzimidazole-5-[N-(2-amidinoethyl]carboxyamide hydrochloride

0.33 g (0.83 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-(2-amidinoethyl)]carboxyamide hydrochloride was suspended in a mixed solvent of DMF and methanol, and catalytic hydrogenation was then carried out in the presence of 10% Pd/C as a catalyst to lead the above-mentioned compound to a corresponding amino compound. This DMF solution was stirred under ice cooling and under a nitrogen gas stream, and a methylene chloride solution of 4-[4-[N,N-bis(2-chloroethyl)amino]phenyl]butyryl chloride {which was prepared by adding 0.55 g (4.6 mmols, 5.0 eq.) of thionyl chloride to 0.28 g (0.92 mmol) of chlorambucil, removing thionyl chloride under reduced pressure after 5 minutes, and then carrying out azeotropic distillation with benzene twice} was added dropwise. The temperature of the suspension was returned to room temperature, followed by stirring for 7 hours. Next, the suspension was concentrated under reduced pressure, and the resulting residue was then purified through silica gel column chromatography (ethyl acetate/IPA/water = 6/2/1), and then solidified with ethanol to obtain 0.23 g (0.36 mmols, 43.4%) of the desired compound in the state of light yellowish white crystals.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.31 (s, 1H), 9.09 (s, 2H), 8.98 (t, 1H), 8.68 (s, 1H), 8.66 (s, 2H), 8.27 (s, 1H), 7.97 (d, 2H), 7.78 (d, 1H), 7.70 (d, 1H), 7.58 (t, 1H), 7.07 (d, 2H), 6.68 (d, 2H), 3.65 (m, 2H), 2.72 (t, 2H), 2.55 (m, 2H), 2.40 (t, 2H), 1.89 (m, 2H)

IR (KBr) cm^{-1}: 3064, 1690, 1519, 1310, 1245, 810, 723

Elemental analysis: ($C_{31}H_{35}Cl_2N_7O_2 \cdot 2HCl \cdot 3H_2O$)				
Calcd.:	C:50.62,	H:5.89,	N:13.33,	Cl:19.28
Found:	C:50.36,	H:5.61,	N:12.77,	Cl:19.76

Example 2 (Compound 2)

1H-2-[4-[[4-[4-[N,N-bis(2-chloroethyl)amino]phenyl]butyryl]amino]phenyl]benzimidazole-5-[N-(2-amidinoethyl]carboxyamide hydrochloride

Reaction 1

Methyl 3,4-diaminobenzoate

3.0 g of 3,4-diaminobenzoic acid was suspended in methanol, and 1.86 ml of thionyl chloride was then added dropwise. Afterward, thionyl chloride was further added as much as 0.5 ml twice, and the suspension was then heated under reflux for 11 hours. Next, thionyl chloride and methanol were distilled off, and the residue was dissolved in methylene chloride, washed with a 0.5N aqueous sodium hydroxide solution and a saturated sodium chloride solution, and then dried over sodium sulfate. After the solvent was distilled off, the residue was sludged with n-hexane to obtain 3.04 g (93%) of the desired compound.

NMR ($CDCl_3$) δ: 7.47 (dd, 1H), 7.41 (d, 1H), 6.67 (d, 1H), 3.85 (s, 3H)

Reaction 2

Methyl 1H-2-(4-nitrophenyl)benzimidazole-5-carboxylate

In 60 ml of nitrobenzene were dissolved 1.0 g (6.0 mmols) of methyl 3,4-diaminobenzoate and 0.91 g (6.0 mmols) of p-nitrobenzaldehyde (slightly undissolved), and the solution was then heated in an oil bath at 150°C for 39 hours. Next, the solution was cooled with ice water, and the resulting crystals were collected by filtration to obtain 1.54 g (5.2 mmols, 86.5%) of the desired compound in the state of brown crystals.

mp: >280°C

NMR (DMSO-d_6) δ: 8.44 (d, 2H), 8.31 (d, 2H), 8.16 (s, 1H), 7.9-7.7 (m, 2H), 3.9 (s, 3H)

Reaction 3

1H-2-(4-nitrophenyl)benzimidazole-5-carboxylic acid

In 20 ml of methanol was suspended 1.0 g (3.4 mmols) of methyl 1H-2-(4-nitrophenyl)benzimidazole-5-carboxylate, and 20 ml of a 1N aqueous sodium hydroxide solution was then added, followed by heating and stirring at 60°C for 1 hour. Next, an amount corresponding to that of methanol was distilled off under reduced pressure. Water was added to the remaining reaction solution, and the solution was then acidified with 4N hydrochloric acid. Afterward, the resulting crystals were collected by filtration to obtain 0.94 g (3.3 mmols, 97.6%) of the desired compound in the state of yellowish white crystals.

mp: >278°C

NMR (DMSO-d_6) δ: 8.47 (d, 2H), 8.43 (d, 2H), 8.25 (s, 1H), 7.9 (d, 1H), 7.7 (d, 1H)

Reaction 4

1H-2-(4-nitrophenyl)benzimidazole-5-[N-(2-cyanoethyl)]carboxyamide

In 42 ml of DMF was dissolved 0.48 g (1.69 mmols) of 1H-2-(4-nitrophenyl)benzimidazole-5-carboxylic acid, and 0.33 g (2.04 mmols, 1.2 eq.) of CDI was added, followed by stirring at room temperature under a nitrogen gas stream. After 3 hours, the solution was cooled on ice, and 0.14 ml (1.9 mmols, 1.1 eq.) of β-aminopropionitrile was then added.

Next, the solution was stirred for 3 hours at room temperature and allowed to stand overnight. After concentration under reduced pressure, the resulting residue was sludged with methanol to obtain 0.47 g (1.4 mmols, 82.9%) of the desired compound in the state of yellow crystals.

mp: >270°C

NMR ($DMSO-d_6$) δ: 8.94-8.9 (m, 1H), 8.45 (s, 4H), 8.3 (s, 0.5H), 8.1 (s, 0.5H), 7.85 (d, 0.5H), 7.65 (d, 0.5H), 3.54 (q, 2H), 2.82 (t, 2H)

Reaction 5

1H-2-(4-nitrophenyl)benzimidazole-5-[N-(2-amidinoethyl)]carboxyamide hydrochloride

In 10 ml of ethanol was suspended 0.47 g (1.4 mmols) of 1H-2-(4-nitrophenyl)benzimidazole-5-[N-(2-cyanoethyl)]carboxyamide, and a hydrochloric acid gas was blown into the suspension over 30 minutes under ice cooling to saturate the suspension with the gas. Afterward, the suspension was stirred at room temperature for 2 hours to precipitate a solid after it was once dissolved. After concentration under reduced pressure, the resulting residue was sludged with ether, collected by filtration, and then suspended in 15 ml of ethanol. Next, an ammonia gas was blown into the suspension over 2 hours to saturate it with the gas, whereby a solid was precipitated after it was once dissolved. The reaction system was allowed to stand overnight, as it was. Next, methanol and acetone were added to the reaction solution, and the undissolved precipitate was collected by filtration to obtain 0.42 g (1.1 mmols, 77.9%) of the desired compound in the state of yellow crystals.

mp: >279°C

NMR ($DMSO-d_6$) δ: 9.5 (bs, 3H), 8.83 (m, 1H), 8.5 (d, 2H), 8.43 (s, 1H), 8.24 (s, 1H), 7.83 (d, 1H), 7.7 (d, 1H), 3.65 (m, 2H), 2.72 (t, 2H)

Reaction 6

1H-2-[4-[[4-[4-[N,N-bis(2-chloroethyl)amino]phenyl]butyryl]amino]phenyl]benzimidazole-5-[N-(2-amidinoethyl]carboxyamide hydrochloride

0.24 g (3.3 mmols) of thionyl chloride was added to 0.12 g (0.39 mmol) of chlorambucil, and the mixture was then stirred at room temperature for 5 minutes. Next, thionyl chloride was distilled off under reduced pressure and further removed by doing azeotropic distillation with benzene twice, and methylene chloride was then added. This solution was added to a DMF solution containing 0.54 mmol of 1H-2-(4-aminophenyl)benzimidazole-5-[N-(2-amidinoethyl]carboxyamide hydrochloride (which was obtained by subjecting the nitro compound of Reaction 5 to catalytic hydrogenation using 10% Pd/C as a catalyst) under ice cooling, and the solution was stirred at room temperature for 5 hours and then allowed to stand overnight. Next, a formed solid was removed by filtration, and the filtrate was then concentrated under reduced pressure. The resulting residue was purified through silica gel column chromatography (methylene chloride/methanol/acetic acid = 80/20/1), and then crystallized from ether, thereby obtaining 71 mg (0.11 mmols, 20.4%) of the desired compound in the state of light brown crystals.

mp: Decomposed from 209°C

NMR ($DMSO-d_6$) δ: 10.4 (s, 1H), 9.07 (s, 2H), 8.97 (m, 1H), 8.64 (s, 2H), 8.29 (d, 2H), 8.24 (s, 1H), 7.95 (d, 1H), 7.87 (d, 2H), 7.77 (d, 1H), 7.07 (d, 2H), 6.67 (d, 2H), 3.70 (s, 8H), 3.64 (m, 2H), 2.71 (m, 2H), 2.39 (m, 2H), 1.88 (m, 2H)

IR (KBr) cm^{-1}: 3100, 1686, 1519, 1322, 1258, 1193, 843, 740

Elemental analysis: ($C_{31}H_{35}Cl_2N_7O_2 \cdot 2HCl \cdot 3H_2O$)				
Calcd.:	C:50.62,	H:5.89,	N:13.33,	Cl:19.28
Found:	C:50.68,	H:5.48,	N:13.63,	Cl:18.88

Example 3 (Compound 90)

2-[3-(formylamino)phenyl]benzimidazole-5-[N-(2-amidinoethyl]carboxyamide hydrochloride

Reaction 1

1H-2-(3-nitrophenyl)benzimidazole-5-[N-(2-amidinoethyl)]carboxyamide hydrochloride

In 40 ml of ethanol was suspended 1.08 g (3.22 mmols) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-(2-cyanoethyl)]carboxyamide, and a hydrochloric acid gas was blown into the suspension over 30 minutes under ice cooling to saturate the suspension with the gas. Next, the temperature of the suspension was returned to room temperature, and after stirring for 3 hours, the suspension was concentrated under reduced pressure. The resulting residue was decanted twice with ether and then suspended in 40 ml of ethanol, and an ammonia gas was blown thereinto under ice cooling over 50 minutes to saturate the suspension with the ammonia gas. Afterward, the temperature of the suspension was returned to room temperature, and the suspension was stirred for 3 hours and then allowed to stand overnight. Next, the suspension was concentrated under reduced pressure, and the resulting residue was then sludged with methanol/acetone. The resulting solid was purified through silica gel column chromatography (ethyl acetate/IPA/water = 5/2/1), and then sludged with methylene chloride and IPA to obtain 0.67 g (1.72 mmols, 53.5%) of the desired compound in the state of creamy crystals.

mp: >270°C

NMR (DMSO-d_6) δ: 9.10-9.06 (m, 3H), 8.88 (t, 0.5H), 8.79 (t, 0.5H), 8.71 (bs, 3H), 8.35 (d, 1H), 8.31 (s, 0.5H), 8.15 (s, 0.5H), 7.88 (t, 1H), 7.88 (d, 1H), 7.75 (d, 0.5H), 7.63 (d, 1H), 3.65 (q, 2H), 2.73 (t, 2H)

Reaction 2

1H-2-[3-(formylamino)phenyl]benzimidazole-5-[N-(2-amidinoethyl)]carboxyamide hydrochloride

In a mixed solvent of 8 ml of DMF and 8 ml of methanol was suspended 0.65 g (1.67 mmols) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-(2-amidinoethyl)]carboxyamide hydrochloride, and catalytic hydrogenation was then carried out by using 0.28 g of 10% Pd/C as a catalyst. Next, the suspension was concentrated under reduced pressure, and to an 1/2 amount (a solution containing about 4 ml of DMF) of the concentrated suspension, a formylimidazole-THF solution [which was prepared by adding 0.16 ml (4.2 mmols) of formic acid to a mixture of 0.67 g (4.1 mmols) of CDI and 12 ml of THF, and then stirring the solution at room temperature in a nitrogen atmosphere for 1 hour] was added dropwise under ice cooling in a nitrogen atmosphere, and the temperature of the suspension was returned to room temperature. Afterward, the suspension was stirred for 5 hours and then allowed to stand overnight. Next, the suspension was concentrated under reduced pressure, and the resulting residue was purified through silica gel column chromatography (ethyl acetate/IPA/H_2O = 6/2/1) and further purified preparative TLC (ethyl acetate/IPA/H_2O = 5/2/1) twice, and then solidified with IPA-ether to obtain 17 mg (0.044 mmol, 5.3%) of the desired compound in the state of yellow amorphous powder.

NMR (DMSO-d_6) δ: 10.31 (s, 1H), 9.09 (s, 2H), 8.98 (t, 1H), 8.68 (s, 1H), 8.66 (s, 2H), 8.27 (s, 1H), 7.97 (d, 2H), 7.78 (d, 1H), 7.70 (d, 1H), 7.58 (t, 1H), 7.07 (d, 2H), 6.68 (d, 2H), 3.65 (m, 2H), 2.72 (t, 2H), 2.55 (m, 2H), 2.40 (t, 2H), 1.89 (m, 2H)

IR (KBr) cm^{-1}: 3064, 1690, 1519, 1310, 1245, 810, 723

Elemental analysis: ($C_{31}H_{35}Cl_2N_7O_2 \cdot 2HCl \cdot 3H_2O$)				
Calcd.:	C:50.62,	H:5.89,	N:13.33,	Cl:19.28
Found:	C:50.36,	H:5.61,	N:12.77,	Cl:19.76

Example 4 (Compound 45)

2-[4-(formylamino)phenyl]benzimidazole-5-[N-(2-amidinoethyl]carboxyamide hydrochloride

Reaction 1

1H-2-(4-aminophenyl)benzimidazole-5-[N-(2-amidinoethyl)]carboxyamide hydrochloride

In a mixed solvent of 4 ml of DMF and 4 ml of methanol was dissolved 0.42 g (1.08 mmols) of 1H-2-(4-nitrophenyl)benzimidazole-5-[N-(2-amidinoethyl)]carboxyamide hydrochloride, and catalytic hydrogenation was then carried out by using 0.18 g of 10% Pd/C as a catalyst. After the removal of the catalyst, methanol was distilled off, and the half of the solution containing DMF was taken out and used in the next reaction.

Reaction 2

1H-2-[4-(formylamino)phenyl]benzimidazole-5-[N-(2-amidinoethyl)]carboxyamide hydrochloride

A solution, which had been prepared by adding 0.10 ml (2.65 mmols) of formic acid to 4 ml of a THF solution containing 0.44g (2.7 mmols) of CDI and then stirring the solution at room temperature for 30 minutes under a nitrogen gas stream, was added dropwise to a DMF solution containing 0.54 mmol of 1H-2-(4-aminophenyl)benzimidazole-5-[N-(2-amidinoethyl)]carboxyamide hydrochloride under ice cooling under a nitrogen gas stream with stirring. Afterward, the temperature of the solution was returned to room temperature, followed by stirring for 6.5 hours. Next, the solution was concentrated under reduced pressure, and the resulting residue was purified through reversed phase silica gel column chromatography (ODS, water/methanol = 50%), and then crystallized from ethanol-ether to obtain 36 mg (0.093 mmol, 17.2%) of the desired compound in the state of white crystals.

mp: 220-229°C

NMR (DMSO-d_6, 80°C) δ: 10.80 (s, 1H), 9.06 (bs, 2H), 8.80 (bs, 3H), 8.40 (s, 1H), 8.17 (d, 1H), 8.12 (s, 1H), 7.93 (d, 2H), 7.72 (d, 1H), 6.83 (d, 2H), 3.45 (m, 2H), 2.44 (m, 2H)

IR (KBr) cm^{-1}: 3422, 1648, 1606, 1499, 1400, 1195, 840

Example 5 (Compound 44)

1H-2-[4-(formylamino)phenyl]benzimidazole-5-[N-[3-(dimethylamino)propyl]]carboxyamide

Reaction 1

1H-2-(4-nitrophenyl)benzimidazole-5-[N-[3-(dimethylamino)propyl]]carboxyamide

In 50 ml of DMF was dissolved 0.50 g (1.77 mmols) of 1H-2-(4-nitrophenyl)benzimidazole-5-carboxylic acid, and 0.34 g (2.1 mmols, 1.2 eq.) of CDI was added, followed by stirring at room temperature in a nitrogen atmosphere. After 4 hours, the solution was cooled on ice. Then, 0.24 ml (1.9 mmols, 1.1 eq.) of N,N-dimethyl-1,3-propanediamine was added to the solution, and the temperature of the solution was returned to room temperature and the solution was then allowed to stand overnight. After the completion of the reaction had been confirmed, the solution was concentrated under reduced pressure, and the resulting residue was sludged with methanol to obtain 0.52 g (1.42 mmols, 80.2%) of the desired compound in the state of yellowish white crystals.

mp: 261-265°C

NMR (DMSO-d_6) δ: 8.57 (t, 1H), 8.45 (s, 4H), 8.17 (s, 1H), 7.80 (d, 1H), 7.71 (d, 1H), 3.33 (q, 2H), 2.30 (t, 2H), 1.70 (m, 2H)

Reaction 2

1H-2-[4-(formylamino)phenyl]benzimidazole-5-[N-[3-(dimethylamino)propyl]]carboxyamide

In a mixed solvent of 6 ml of DMF and 4 ml of methanol was suspended 0.18 g (0.49 mmol) of 1H-2-(4-nitrophenyl)benzimidazole-5-[N-[3-(dimethylamino)propyl]]carboxyamide, and catalytic hydrogenation was then carried out by the use of 10% Pd/C as a catalyst to lead it to a corresponding amino compound. This compound was dissolved in 8 ml of DMF and then ice-cooled, and a formylimidazole/ THF solution [0.38 g (2.34 mmols) of CDI, prepared from 88 μl of formic acid and 4 ml of THF] was added dropwise under a nitrogen gas stream. Next, the temperature of the solution was returned to room temperature, and the solution was stirred for 7.5 hours and then allowed to stand overnight. After

concentration under reduced pressure, the resulting residue was purified through alumina column chromatography (ICN, Almina N, adjusted to Grade 3 by adding water) (chloroform/methanol = 8-10%), and then crystallized from ethanol-ether to obtain 0.11 g (0.30 mmol, 64.0%) of the desired compound in the state of yellowish white crystals.

mp: 126-132°C

NMR (DMSO-d_6) δ: 10.43 (s, 1H), 8.45 (bs, 1H), 8.35 (s, 0.5H), 7.98 (s, 0.5H), 8.14 (d, 2H), 7.77 (d, 2H), 7.68 (bs, 1H), 7.54 (d, 0.5H), 7.40 (d, 0.5H), 3.32 (m, 2H), 2.29 (t, 2H), 2.16 (s, 3H)

IR (KBr) cm^{-1}: 3379, 2949, 1692, 1608, 1545, 1290, 844, 737

Elemental analysis: ($C_{20}H_{23}N_5O_2 \cdot 0.5H_2O$)			
Calcd.:	C:64.15,	H:6.46,	N:18.70
Found:	C:64.25,	H:6.67,	N:18.50

Example 6 (Compound 29)

1H-2-[4-[[4-[4-[N,N-bis(2-chloroethyl)amino]phenyl]butyryl]amino]phenyl]benzimidazole-5-[N-[3-(dimethylamino)propyl]]carboxyamide

In a mixed solvent of 6 ml of DMF and 4 ml of methanol was suspended 0.15 g (0.41 mmol) of 1H-2-(4-nitrophenyl)benzimidazole-5-[N-[3-(dimethylamino)propyl]]carboxyamide, and catalytic hydrogenation was then carried out by the use of 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, this compound was dissolved in 8 ml of DMF, and 0.12 g (0.39 mmol, 0.96 eq.) of chlorambucil and 61 mg (0.45 mmol, 1.1 eq.) of HOBt were then added. Afterward, 96 mg (0.47 mmol, 1.1 eq.) of DCC was further added under ice cooling in a nitrogen atmosphere, and the temperature of the solution was returned to room temperature. Next, the solution was stirred for 7 hours and then allowed to stand overnight. Since the reaction did not complete, a solution prepared by the following procedure was added to the reaction system, followed by stirring for 10 hours. The above-mentioned solution was prepared by reacting 0.12 g (0.39 mmol, 0.95 eq.) of chlorambucil with 0.24 g of thionyl chloride at room temperature for 5 minutes, removing thionyl chloride by azeotropic distillation with benzene, and then dissolving the thionyl chloride-free material in methylene chloride. After the completion of the reaction had been confirmed, the resulting solid was removed by filtration, and the filtrate was then concentrated under reduced pressure. Afterward, the resulting residue was purified through alumina column chromatography (ICN, Almina N, methylene chloride/methanol = 2%), and then crystallized from ether to obtain 0.13 g (0.21 mmol, 51.2%) of the desired compound in the state of white crystals.

mp: 200-210°C (decomposed)

NMR (DMSO-d_6) δ: 10.14 (s, 1H), 8.54-8.5 (m, 1H), 8.15 (s, 0.5H), 8.00 (s, 0.5H), 8.10 (d, 1H), 7.80 (d, 2H), 7.70-7.51 (m, 2H), 7.12 (d, 2H), 6.71 (d, 2H), 3.70 (s, 8H), 3.34-3.36 (m, 2H), 2.53 (m, 2H), 2.41-2.33 (m, 4H), 2.17 (s, 6H), 1.92 (m, 2H), 1.70 (m, 2H)

IR (KBr) cm_{-1}: 2947, 1615, 1519, 1311, 1252, 846

Elemental analysis: ($C_{33}H_{40}Cl_2N_6O_2$)			
Calcd.:	C:63.56,	H:6.46,	N:13.48
Found:	C:63.10,	H:6.53,	N:13.15

Example 7 (Compound 1001)

1H-2-[4-(guanidinoacetylamino)phenyl]benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide dihydrochloride

Reaction 1

1H-2-(4-nitrophenyl)benzimidazole-5-[N-[4-[N,N-bis (2-chloroethyl)amino]phenyl]]carboxyamide

In 8 ml of DMF were suspended 0.26 g (0.96 mmol) of 1H-2-(4-nitrophenyl)benzimidazole-5-carboxylic acid and 0.27 g (0.95 mmol) of N,N-bis(2-chloroethyl)-1,4-phenylenediamine hydrochloride, and the solution was then stirred under a nitrogen gas stream, while cooled on ice. Next, 0.40 ml (2.87 mmols, 3.0 eq.) of triethylamine and 0.22 ml (1.45 mmols, 1.5 eq.) of DECP were added in this order, and the solution was stirred for 3 hours and then allowed to stand overnight, as it was. After concentration under reduced pressure, the resulting residue was sludged with methanol to obtain 0.33 g (0.66 mmol, 69.7%) of the desired compound in the state of light brown crystals.

mp: >250°C

NMR (DMSO-d_6) δ: 10.11 (s, 0.5H), 10.05 (s, 0.5H), 8.46 (s, 4H), 8.42 (s, 0.5H), 8.17 (s, 0.5H), 7.95-7.84 (m, 1.5H), 7.68 (d, 0.5H), 6.77 (d, 2H), 6.64 (d, 2H), 3.74 (s, 8H)

Reaction 2

1H-2-[4-(guanidinoacetylamino)phenyl]benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide dihydrochloride

0.12 ml of 1N hydrochloric acid was added to a solution conposed of a mixed solvent of DMF and methanol and 50 mg (0.10 mmol) of 1H-2-(4-nitrophenyl)benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide. Then the mixture was subjected to catalytic hydrogenation in the presence of 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 20 µl (0.14 mmol, 1.4 eq.) of triethylamine, 46 mg (0.30 mmol, 3.0 eq.) of guanidineacetic acid hydrochloride and 62 mg (0.30 mmol, 3.0 eq.) of DCC were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was stirred for 2 hours and then allowed to stand overnight. After the removal of a formed solid by filtration, the filtrate was then concentrated under reduced pressure. The resulting residue was subjected to gel filtration (Sephadex LH-20, methanol), and 4N hydrochloric acid and dioxane were added to the eluted fraction. Next, the solution was concentrated and then sludged with methanol to obtain 10 mg (0.016 mmol, 15.6%) of the desired compound in the state of white crystals.

mp: 215-227°C (decomposed)

NMR (DMSO-d_6) δ: 10.30 (s, 1H), 8.35 (d, 2H), 8.32 (s, 1H), 8.05 (d, 1H), 7.93 (d, 2H), 7.83 (d, 1H), 7.63 (d, 3H), 7.50-7.20 (bs, 4H), 6.78 (d, 2H), 4.16 (d, 2H), 3.74 (s, 8H)

IR (KBr) cm^{-1}: 3332, 1652, 1602, 1516, 1328, 737

Example 8 (Compound 1010)

1H-2-[4-(guanidinoacetylamino)phenyl]benzimidazole-5-[N-[3-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide

Reaction 1

m-[N,N-bis(2-hydroxyethyl)amino]nitrobenzene

In 36 ml of 30% acetic acid was dissolved 5.0 g (36.2 mmols) of m-aminonitrobenzene, and 22.9 ml of ethylene oxide was further added under ice cooling, followed by stirring at room temperature overnight. After extraction with ethyl acetate, the ethyl acetate layer was dried over sodium sulfate and concentrated. The resulting residue was sludged with ether to obtain 5.21 g (23.0 mmols, 63.6%) in the state of yellow crystals.

mp: 98.5-100°C

NMR (DMSO-d_6) δ: 7.51 (d, 1H), 7.51 (s, 1H), 7.32 (t, 1H), 6.99 (d, 1H), 3.89 (t, 4H), 3.73 (bs, 2H), 3.65 (t, 4H)

Reaction 2

3-[N,N-bis(2-chloroethyl)amino]nitrobenzene

In 25 ml of toluene was suspended 2.5 g (11.0 mmols) of 3-[N,N-bis(2-hydroxyethyl)amino]nitrobenzene, and 10.2 g (85.7 mmols, 7.8 eq.) of thionyl chloride was added under ice cooling. Next, the suspension was heated and stirred for 5 hours in an oil bath at 70°C. After concentration under reduced pressure, the solution was extracted with ethyl acetate after addition of water. After drying over anhydrous sodium sulfate, the extracted layer was concentrated under reduced pressure, and the resulting residue was then sludged with ether to obtain 2.67 g (10.1 mmols, 92.2%) of the desired compound in the state of yellow crystals.

mp: 112-113°C

NMR ($CDCl_3$) δ: 7.61 (d, 1H), 7.50 (t, 1H), 7.39 (t, 1H), 6.98 (d, 1H), 3.83 (t, 4H), 3.68 (t, 4H)

Reaction 3

N,N-bis(2-chloroethyl)-1,3-phenylenediamine hydrochloride

In 35 ml of concentrated hydrochloric acid was dissolved 2.0 g (7.6 mmols) of 3-[N,N-bis(2-chloroethyl)amino]nitrobenzene, and 6.9 g (30.6 mmols, 4.0 eq.) of stannic chloride (II) dihydrate was added, followed by stirring for 1 hour in an oil bath at 100°C. Next, the solution was allowed to stand until its temperature lowered to room temperature, and then diluted with water. The solution was basified with concentrated ammonia water, extracted with ethyl acetate twice, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure. Afterward, 4N hydrochloric acid and dioxane were added to the resulting residue, and after concentration, the residue was crystallized from a small amount of methanol-ether to obtain 1.97 g (7.3 mmols, 96.1%) of the desired compound in the state of yellow crystals.

mp: 195-201°C

NMR (DMSO-d_6) δ: 10.30-9.80 (bs, 3H), 7.27 (t, 1H), 6.76 (d, 1H), 6.73 (s, 1H), 6.63 (d, 1H), 3.74 (s, 8H)

Reaction 4

1H-2-(4-nitrophenyl)benzimidazole-5-[N-3-[N,N-bis(2-chloroethyl)amino]phenyl]carboxyamide

In 10 ml of DMF were dissolved 0.30 g (1.06 mmols) of 1H-2-(4-nitrophenyl)benzimidazole-5-carboxylic acid and 0.29 g (1.08 mmols, 1.0 eq.) of N,N-bis(2-chloroethyl)-1,3-phenylenediamine hydrochloride, and the solution was then stirred under a nitrogen gas stream, while cooled on ice. Next, 0.44 ml (3.15 mmols, 3.0 eq.) of triethylamine and 0.24 ml (1.58 mmols, 1.5 eq.) of DECP were added in this order, and the solution was stirred for 9 hours and then allowed to stand overnight, as it was. After concentration under reduced pressure, the resulting residue was purified through silica gel column chromatography (chloroform/methanol = 2-4%) and then sludged with ethanol to obtain 0.23 g (0.46 mmol, 43.5%) of the desired compound in the state of yellowish white crystals.

mp: 213-217°C

NMR (DMSO-d_6) δ: 10.20 (s, 0.5H), 10.11 (s, 0.5H), 8.47 (s, 4H), 8.43 (s, 1H), 8.16 (s, 0.5H), 7.92 (d, 0.5H), 7.86 (s, 0.5H), 7.71 (d, 0.5H), 7.31-7.15 (m, 3H), 6.51 (d, 1H), 3.80-3.74 (m, 8H)

Reaction 5

1H-2-[4-(guanidinoacetylamino)phenyl]benzimidazole-5-[N-[3-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide dihydrochloride

In DMF-methanol, 50 mg (0.10 mmol) of 1H-2-(4-nitrophenyl)benzimidazole-5-[N-3-[N,N-bis(2-chloroethyl)amino]phenyl]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 44 mg (0.29 mmol, 2.9 eq.) of guanidineacetic acid hydrochloride and 59 mg (0.29 mmol, 2.9 eq.) of DCC were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was stirred for 5 hours and then allowed to stand overnight. After the removal of a formed solid by filtration, the filtrate was then concentrated under reduced pressure. Next, DMF was added to the residue, and the resulting crystals were removed by filtration. The concentrated residue of the filtrate was subjected to gel filtration (Sephadex LH-20, methanol), and 4N hydrochloric acid and dioxane were added to the eluted fraction, followed by concentration. The concentrated material was crystallized from ether to obtain 31 mg (0.048 mmol, 48.0%) of the desired compound in the state of light yellowish white crystals.

mp: 200-210°C

NMR (DMSO-d_6) δ: 10.27 (s, 1H), 9.41 (s, 1H), 8.33 (d, 2H), 8.30 (s, 1H), 7.96 (d, 1H), 7.90 (d, 2H), 7.80 (d, 1H), 7.64 (t, 1H), 7.50-7.15 (m, 7H), 6.51 (d, 1H), 4.16 (d, 2H), 3.76 (m, 8H)

IR (KBr) cm^{-1}: 3339, 1654, 1604, 1542

Example 9 (Compound 2001)

1H-2-[3-(guanidinoacetylamino)phenyl]benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide dihydrochloride

Reaction 1

1H-2-(3- nitrophenyl)benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide

In 12 ml of DMF were dissolved 0.30 g (1.06 mmols) of 1H-2-(3-nitrophenyl)benzimidazole-5-carboxylic acid and 0.29 g (1.08 mmols, 1.0 eq.) of N,N-bis(2-chloroethyl)-1,4-phenylenediamine hydrochloride, and the solution was then stirred under a nitrogen gas stream while cooled on ice. Next, 0.45 ml (3.23 mmols, 3.0 eq.) of triethylamine and 0.24 ml (1.58 mmols, 1.5 eq.) of DECP were added in this order, and the solution was stirred for 3 hours and then allowed to stand overnight, as it was. After concentration under reduced pressure, methanol was added to the resulting residue, and the solution was then allowed to stand for 3 hours. Next, the resulting solid was collected by filtration to obtain 0.45 g (0.90 mmol, 85.2%) of the desired compound in the state of ocherous powder.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.06 (bs, 1H), 9.06 (s, 1H), 8.66 (d, 1H), 8.38 (d, 1H), 8.38 (s, 0.5H), 8.16 (s, 0.5H), 7.90 (t, 1H), 7.93-7.70 (m, 2H), 7.63 (d, 2H), 6.77 (d, 2H), 3.74 (s, 8H)

Reaction 2

1H-2-[3-(guanidinoacetylamino)phenyl]benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide dihydrochloride

In DMF-methanol, 0.15 g (0.30 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.14 g (0.91 mmols, 3.0 eq.) of guanidineacetic acid hydrochloride and 0.19 g (0.92 mmol, 3.0 eq.) of DCC were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was stirred for 9 hours and then allowed to stand overnight. After the removal of a formed solid by filtration, the filtrate was concentrated under reduced pressure. Next, the residue was subjected to gel filtration (Sephadex LH-20, methanol), and 4N hydrochloric acid and dioxane were added to the eluted fraction, followed by concentration. The concentrated solution was crystallized from methanol, thereby obtaining 0.13 g (0.20 mmol, 67.7%) of the desired compound in the state of light yellowish white crystals.

mp: >250°C

NMR (DMSO-d_6) δ: 10.71 (s, 1H), 10.23 (s, 1H), 8.65 (s, 1H), 8.31 (s, 1H), 8.01 (d, 2H), 7.82-7.77 (m, 2H), 7.64 (m, 4H), 7.60-7.20 (bs, 4H), 6.77 (d, 2H), 4.16 (d, 2H), 3.74 (s, 8H)

IR (KBr) cm^{-1}: 3310, 1652, 1517

Elemental analysis: ($C_{27}H_{28}Cl_2N_8O_2 \cdot 2HCl \cdot H_2O$)				
Calcd.:	C:49.25,	H:4.90,	N:17.02,	Cl:21.54
Found:	C:49.30,	H:4.70,	N:16.91,	Cl:21.61

Example 10 (Compound 2004)

1H-2-[3-(guanidinoacetylamino)phenyl]benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]] carboxyamide dihydrochloride

Reaction 1

1H-2-(3- nitrophenyl)benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide

In 30 ml of DMF were dissolved 3.1 g (11 mmols) of 1H-2-(3-nitrophenyl)benzimidazole-5-carboxylic acid and 3.1 g (11 mmols, 1.0 eq.) of 3-methyl-4-[N,N-bis(2-chloroethyl)amino]aniline hydrochloride, and the solution was then stirred under a nitrogen gas stream while cooled on ice. Next, 2.5 ml (16 mmols, 1.5 eq.) of DECP and 4.6 ml (33 mmols, 3.0 eq.) of triethylamine were added in this order, and the solution was stirred at 0°C for 30 minutes and at room temperature for 3 hours and then allowed to stand overnight. After the reaction solution was concentrated under reduced pressure, the resulting residue was dissolved in acetone. Next, silica gel was added thereto, and the solution was evaporated to dryness under reduced pressure and then purified through silica gel column chromatography (chloroform/methanol = 8/2). After the solvents were distilled off under reduced pressure, the resulting residue was sludged with chloroform and a small amount of methanol. Next, the resulting powder was collected by filtration and then washed with methanol, thereby obtaining 4.2 g (yield = 75%) of ocherous powder.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.22 (s, 0.5H), 10.16 (s, 0.5H), 9.06 (s, 1H), 8.66 (d, 1H), 8.42-8.36 (m, 1.5H), 8.17 (s, 0.5H), 7.93-7.62 (m, 5H), 7.24 (d, 1H), 3.57 (t, 4H), 3.39 (t, 4H), 2.31 (s, 3H)

Reaction 2

1H-2-[3-(guanidinoacetylamino)phenyl]benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino] phenyl]]carboxyamide dihydrochloride

In a mixed solvent of 5 ml of DMF and 5 ml of methanol, 0.40 g (0.78 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 281 mg (2.3 mmols, 3 eq.) of guanidineacetic acid hydrochloride and 483 mg (2.3 mmols, 3 eq.) of DCC were added in this order. After stirring at 0°C for 30 minutes, the solution was further stirred at room temperature for 6 hours and then allowed to stand overnight. Next, the resulting precipitate was removed by filtration and the solvents were distilled off from the resulting filtrate under reduced pressured. The resulting residue was purified through silica gel column chromatography (chloroform/methanol = 95/5). After the solvents were distilled off under reduced pressure, the resulting residue was dissolved in methanol, and ether was then added to the solution, so that precipitation occurred again, thereby obtaining 155 mg (yield = 34%) of white powder.

NMR (DMSO-d_6) δ: 10.83 (s, 1H), 10.44 (s, 1H), 8.68 (s, 1H), 8.37 (s, 1H), 8.09 (d, 2H), 7.89 (d, 1H), 7.82 (d, 1H), 7.73-7.63 (m, 3H), 7.44 (bs, 4H), 7.25 (d, 1H), 4.19 (d, 2H), 3.57 (t, 4H), 3.37 (t, 4H), 2.31 (s, H)

IR (KBr) cm^{-1}: 3348, 1665, 1504, 1308, 1263, 884

Example 11 (Compound 2006)

1H-2-[3-(guanidinoacetylamino)phenyl]benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino] phenyl]]carboxyamide dihydrochloride

Reaction 1

1H-2-(3- nitrophenyl)benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide

In 7 ml of DMF was dissolved 0.60 g (2.12 mmols) of 1H-2-(3-nitrophenyl)benzimidazole-5-carboxylic acid, and the solution was then stirred under a nitrogen gas stream while cooled on ice. Afterward, 2.45 mmols of 3-chloro-4-[N,N-bis(2-chloroethyl)amino]aniline hydrochloride {which was synthesized from 0.73 g (2.45 mmols) of a corresponding nitro compound by catalytic hydrogenation} and 0.89 ml (6.38 mmols, 3.0 eq.) of triethylamine were added in this order, and 5 ml of DMF was further added. Next, 0.48 ml (3.16 mmols, 1.5 eq.) of DECP was additionally added, the solution was stirred for 6 hours and then allowed to stand overnight, as it was. After concentration under reduced pressure, the resulting residue was purified through silica gel column chromatography (chloroform/methanol = 4%) and then crystallized from ethyl acetate. According to confirmation by NMR, the introduction of triethylamine hydrochloride was observed,

and therefore, together with the resulting filtrate, the crystals were subjected to gel filtration (Sephadex LH-20, methanol). Next, solidification was accomplished with ether, thereby obtaining 0.48 g (0.90 mmol, 42.0%) of the desired compound in the state of ocherous solid.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.39 (s, 1H), 9.06 (s, 1H), 8.66 (d, 1H), 8.38 (d, 1H), 8.32 (s, 1H), 8.03 (d, 1H), 7.93-7.87 (m, 2H), 7.76 (d, 1H), 7.73 (dd, 1H), 7.37 (d, 1H), 3.64-3.48 (m, 8H)

Reaction 2

1H-2-[3-(guanidinoacetylamino)phenyl]benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino] phenyl]]carboxyamide dihydrochloride

In DMF-methanol, 0.21 g (0.40 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.18 g (1.17 mmols, 3.0 eq.) of guanidineacetic acid hydrochloride and 0.24 g (1.16 mmols, 3.0 eq.) of DCC were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was stirred for 2 hours and then allowed to stand overnight. After the removal of a formed solid by filtration, the filtrate was concentrated under reduced pressure. Next, the residue was subjected to gel filtration (Sephadex LH-20, methanol), and 4N hydrochloric acid and dioxane were added to the eluted fraction, followed by concentration. The concentrated material was solidified with ether, thereby obtaining 0.15 g (0.22 mmol, 55.7%) of the desired compound in the state of light yellowish white power.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.78 (s, 1H), 10.63 (s, 1H), 8.67 (s, 1H), 8.37 (s, 1H), 8.04 (m, 2H), 7.88-7.63 (m, 5H), 7.60-7.25 (bs, 4H), 7.37 (d, 1H), 4.18 (d, 2H), 3.62 (t, 4H), 3.51 (t, 4H)

IR (KBr) cm^{-1}: 3313, 1673, 1498, 1385, 1307, 1252

Elemental analysis: ($C_{27}H_{27}Cl_3N_8O_2 \cdot 2HCl \cdot 5H_2O$)			
Calcd.:	C:42.40,	H:5.14,	N:14.65
Found:	C:42.48,	H:4.73,	N:14.67

Example 12 (Compound 2011)

1H-2-[3-[[4-(guanidino)butyryl]amino]phenyl]benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino)phenyl]]carboxyamide dihydrochloride

In DMF-methanol, 0.20 g (0.40 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.22 g (1.21 mmols, 3.0 eq.) of guanidinobutyric acid hydrochloride and 0.25 g (1.21 mmols, 3.0 eq.) of DCC were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was stirred for 3 hours and then allowed to stand overnight. After the removal of a formed solid by filtration, the filtrate was concentrated under reduced pressure. Next, the residue was purified through silica gel column chromatography (ethyl acetate/IPA/water = 6/2/1) and then subjected to gel filtration (Sephadex LH-20, methanol), and 4N hydrochloric acid and dioxane were added to the eluted fraction, followed by concentration. The concentrated material was solidified with ether, thereby obtaining 0.10 g (0.15 mmol, 37.5%) of the desired compound in the state of yellow power (hygroscopic).

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.59 (s, 1H), 10.32 (s, 1H), 8.69 (s, 1H), 8.36 (s, 1H), 8.10 (d, 1H), 8.05 (d, 1H), 7.95 (t, 1H), 7.87 (d, 1H), 7.79 (d, 1H), 7.65 (m, 3H), 7.50-7.00 (bs, 4H), 6.77 (d, 2H), 3.74 (s, 8H), 3.18 (q, 2H), 1.85 (m, 2H)

IR (KBr) cm^{-1}: 3164, 1655, 1517, 1330, 1247, 1182

Elemental analysis: ($C_{29}H_{32}Cl_2N_8O_2 \cdot 2HCl \cdot 3.3H_2O$)				
Calcd.:	C:47.85,	H:5.62,	N:15.39,	Cl:19.48
Found:	C:47.95,	H:5.49,	N:15.10,	Cl:19.35

Example 13 (Compound 2014)

1H-2-[3-[[4-(guanidino)butyryl]amino]phenyl]benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide dihydrochloride

In a mixed solvent of 5 ml of DMF and 5 ml of methanol, 0.40 g (0.78 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C (wet) as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 426 mg (2.4 mmols, 3 eq.) of 4-guanidinobutyric acid hydrochloride and 450 mg (2.4 mmols, 3 eq.) of EDCI were added in this order. Afterward, the solution was stirred at 0°C for 30 minutes and further at room temperature for 3 hours, and then allowed to stand overnight. The solvents were distilled under reduced pressure, and the resulting residue was then purified through silica gel column chromatography (ethyl acetate/ IPA/water = 5/2/1). After the solvents were distilled off under reduced pressure, the resulting residue was dissolved in methanol and then further subjected to gel filtration column chromatography (Sephadex LH-20, methanol). After the solvents were distilled off under reduced pressure, the resulting residue was then sludged with acetone-IPA, thereby obtaining 113 mg (yield 21%) of the desired compound in the state of light yellow power.

NMR (DMSO-d_6) δ: 10.58 (s, 1H), 10.44 (s, 1H), 8.69 (s, 1H), 8.36 (s, 1H), 8.11-7.61 (m, 7H), 7.25 (bs, 4H), 7.25 (d, 1H), 4.03 (q, 1H), 3.57 (t, 4H), 3.37 (t, 4H), 3.26 (q, 2H), 2.50 (t, 2H), 2.31 (s, 3H), 1.84 (m, 2H)

IR (KBr) cm^{-1}: 3357, 1664, 1504, 1308, 1180, 886

Example 14 (Compound 2016)

1H-2-[3-[[4-(guanidino)butyryl]amino]phenyl]benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide dihydrochloride

In DMF-methanol, 0.31 g (0.58 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.32 g (1.76 mmols, 3.0 eq.) of guanidinobutyric acid hydrochloride and 0.36 g (1.74 mmols, 3.0 eq.) of DCC were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was stirred for 3 hours and then allowed to stand overnight. Since the material did not disappear the next day, 0.11 g 4-guanidinobutyric acid and 0.12 g of DCC were added, followed by stirring for further 2 hours. After the removal of a formed solid by filtration, the filtrate was concentrated under reduced pressure, and the resulting residue was then purified through silica gel column chromatography (ethyl acetate/IPA/water = 6/2/1) and then further subjected to gel filtration (Sephadex LH-20, methanol). Next, 4N hydrochloric acid and dioxane were added to the eluted fraction, followed by concentration. The residue was collected by scraping, thereby obtaining 73 mg (0.10 mmol, 17.9%) of the desired compound in the state of a white solid.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.37 (s, 1H), 10.29 (s, 1H), 8.59 (s, 1H), 8.29 (bs, 1H), 8.03 (s, 1H), 7.88-7.72 (m, 6H), 7.51 (t, 1H), 7.36 (d, 1H), 7.40-6.80 (bs, 4H), 3.62 (m, 4H), 3.50 (m, 4H), 3.19 (m, 2H), 2.46 (m, 2H), 1.84 (m, 2H)

IR (KBr) cm^{-1}: 3178, 1662, 1498, 1393, 1304

Elemental analysis: ($C_{29}H_{31}Cl_3N_8O_2 \cdot 2HCl \cdot 0.5H_2O$)			
Calcd.:	C:48.93,	H:4.81,	N:15.74
Found:	C:48.65,	H:5.16,	N:15.65

Example 15 (Compound 2031)

1H-2-[3-[[4-(dimethylamino)butyryl]amino]phenyl)benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide dihydrochloride

In DMF-methanol, 0.20 g (0.40 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.20 g (1.19 mmols, 3.0 eq.) of 4-dimethylaminobutyric acid hydrochloride and 0.25 g (1.21 mmols, 3.0 eq.) of DCC were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was stirred for 4.5 hours and then allowed to stand overnight. After the removal of a formed solid by filtration, the filtrate was concentrated under reduced pressure, and the resulting residue was then purified through silica gel column chromatography (ethyl acetate/IPA/water = 5/2/1) and then further subjected to gel filtration (Sephadex LH-20, methanol). Since the presence of impurities was observed, the solution was subjected to silica gel column chromatography (ethyl acetate/methanol = 1/1) again and then further subjected to gel filtration (Sephadex LH-20, methanol). Next, solidification was carried out with ether to obtain 89 mg (0.14 mmol, 34.0%) of the desired compound in the state of a light yellowish white solid.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.25 (s, 1H), 10.07 (s, 0.5H), 10.01 (s, 0.5H), 8.59 (s, 0.5H), 8.57 (s, 0.5H), 8.33 (s, 0.5H), 8.12 (s, 0.5H), 7.88-7.48 (m, 7H), 6.77 (d, 2H), 3.74 (s, 8H), 2.75 (m, 2H), 2.54 (s, 6H), 2.45 (s, 6H), 1.90 (m, 2H)

IR (KBr) cm^{-1}: 3214, 1614, 1518, 1328, 1244, 1182

Elemental analysis: ($C_{30}H_{34}Cl_2N_6O_2 \cdot HCl \cdot H_2O$)			
Calcd.:	C:56.65,	H:5.86,	N:13.21
Found:	C:56.60,	H:5.78,	N:13.00

Example 16 (Compound 2091)

1H-2-[3-[[3-(methylthio)propionyl]amino]phenyl]benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide

In a mixed solvent of 5ml of DMF and 3ml of methanol, 0.27 g (0.54 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 70 µl (0.68 mmol, 1.25 eq.) of 3-(methylthio)propionic acid and 0.13 g (0.63 mmol, 1.2 eq.) of DCC were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was stirred for 2.5 hours and then allowed to stand overnight. Since the progress of the reaction stopped the next day, 70 µl (0.68 mmol) of 3-(methylthio)propionic acid and 0.13 g (0.63 mmol) of DCC were added, and the solution was stirred for 10 hours and then allowed to stand overnight. After the removal of a formed solid by filtration, the filtrate was concentrated under reduced pressure, and the resulting residue was then purified through silica gel column chromatography (chloroform/methanol = 4%) and then crystallized from ether, thereby obtaining 0.17 g (0.30 mmol, 55.2%) of the desired compound in the state of light yellowish white crystals.

mp: 142-146°C

NMR (DMSO-d_6) δ: 10.21 (s, 1H), 10.07 (s, 0.4H), 10.01 (s, 0.6H), 8.55 (m, 1H), 8.33 (s, 0.6H), 8.11 (s, 0.4H), 7.87-7.59 (m, 6H), 7.51 (t, 1H), 6.77 (d, 2H), 3.74 (s, 8H), 2.82-2.65 (m, 4H), 2.12 (s, 2H)

IR (KBr) cm^{-1}: 3259, 1644, 1518, 1327, 1248, 1181, 814

Elemental analysis: ($C_{28}H_{29}Cl_2N_6O_2S \cdot H_2O$)			
Calcd.:	C:57.14,	H:5.31,	N:11.90
Found:	C:57.09,	H:5.40,	N:11.67

Example 17 (Compound 2041)

2-[N-[3-[5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]carbamoyl]-1H-benzimidazole-2-yl]phenyl]]carbamoyleth yl-dimethylsulfonium iodide

0.10 g (0.18 mmol) of 1H-2-[3-[[3-(methylthio)propionyl]amino]phenyl]benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was dissolved in a mixture of 0.5 ml of 80% formic acid, 0.25 ml of acetic acid and 0.2 ml of methyl iodide, and the solution was then stirred at room temperature for 9 hours under shading and then allowed to stand overnight. After concentration under reduced pressure, the resulting residue was subjected to gel filtration (Sephadex LH-20, methanol, carried out twice) and then solidified with ether to obtain 47 mg (0.07 mmol, 36.6%) of the desired compound in the state of light yellowish white powder.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.49 (s, 1H), 10.05 (s, 1H), 8.58 (s, 1H), 8.24 (s, 1H), 7.87 (m, 2H), 7.70-7.47 (m, 5H), 6.77 (d, 2H), 3.74 (s, 8H), 3.57 (t, 2H), 3.04 (t, 2H), 2.97 (s, 6H)

IR (KBr) cm^{-1}: 3407, 1645, 1614, 1517, 1328, 1249, 1181

Elemental analysis: ($C_{29}H_{32}Cl_2IN_5O_2S \cdot HCl$)			
Calcd.:	C:46.51,	H:4.44,	N:9.35
Found:	C:46.20,	H:4.24,	N:9.08

Example 18 (Compound 2092)

1H-2-[3-[[3-(methylthio)propionyl]amino]phenyl]benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2- chloroethyl)amino]phenyl]]carboxyamide

In a mixed solvent of 5 ml of DMF and 5 ml of methanol, 0.40 g (0.78 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 281 mg (2.3 mmols, 3 eq.) of 3-(methylthio)propionic acid and 483 mg (2.3 mmols, 3 eq.) of DCC were added in this order. Afterward, the solution was stirred at 0°C for 30 minutes and further at room temperature for 6 hours and then allowed to stand overnight. After the resulting precipitate was removed by filtration, the solvents were distilled off under reduced pressure, and the resulting residue was then purified through silica gel column chromatography (chloroform/methanol = 95/5). After the solvents were distilled off under reduced pressure, the resulting residue was dissolved in methanol, and ether was then added, so that precipitation occurred again, thereby obtaining 155 mg (yield = 34%) of the desired compound in the state of white powder.

NMR (DMSO-d_6) δ: 10.21 (s, 1H), 10.16 (s, 1H), 8.57 (s, 1H), 8.24 (bs, 1H), 7.86 (d, 2H), 7.72-7.49 (m, 5H), 7.24 (d, 1H), 3.57 (t, 4H), 3.36 (t, 4H), 2.80 (t, 2H), 2.68 (t, 2H), 2.37 (s, 3H), 2.12 (s, 3H)

Example 19 (Compound 2044)

2-[N-[3-[5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]carbamoyl]-1H-benzimidazole-2-yl]phenyl]]carbamoylethyl-dimethylsulfonium iodide

0.10 g (0.17 mmol) of 1H-2-[3-[[3-(methylthio)propionyl]amino]phenyl]benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was dissolved in a mixture of 0.6 ml of 80% formic acid, 0.3 ml of acetic acid and 0.5 ml of methyl iodide, and the solution was then stirred at room temperature for 3 hours under shading and then allowed to stand overnight. After concentration under reduced pressure, the resulting residue was purified through gel filtration column chromatography (Sephadex LH-20, methanol, carried out twice). The solvents were distilled off under reduced pressure, and the resulting residue was dissolved in methanol. Afterward, IPA was added to the solution, so that precipitation occurred again, thereby obtaining 58 mg (yield = 47%) of the desired compound in the state of light yellow powder.

NMR (DMSO-d_6) δ: 10.56 (s, 1H), 10.20 (s, 1H), 8.59 (s, 1H), 8.27 (s, 1H), 7.91 (d, 2H), 7.78-7.55 (m, 5H), 7.24 (d, 1H), 3.57 (t, 4H), 3.37 (t, 4H), 3.21 (t, 2H), 3.05 (t, 2H), 2.97 (s, 6H), 2.31 (s, 3H)

IR (KBr) cm-1: 3422, 1654, 1502, 1313, 1118, 885

Example 20 (Compound 2093)

1H-2-[3-[3-(methylthio)propionylamino]phenyl]benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide

In DMF-methanol, 0.21 g (0.40 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.12 ml (1.16 mmols, 2.9 eq.) of 3-methylthiopropionic acid and 0.24 g (1.16 mmols, 2.9 eq.) of DCC were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was stirred for 2 hours and then allowed to stand overnight. After the removal of a formed solid by filtration, the filtrate was concentrated under reduced pressure, and the resulting residue was subjected to silica gel column chromatography (chloroform/ methanol = 4%) and then solidified with ether, thereby obtaining 0.18 g (0.30 mmol, 75.3%) of the desired compound in the state of light yellowish white powder.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.36 (s, 1H), 10.23 (s, 1H), 8.58 (s, 1H), 8.25 (s, 1H), 8.02 (d, 1H), 7.85 (d, 2H), 7.75-7.69 (m, 2H), 7.52 (t, 1H), 7.37 (d, 1H), 3.62 (t, 4H), 3.50 (t, 4H), 2.80 (t, 2H), 2.68 (t, 2H)

Example 21 (Compound 2046)

2-[N-[3-[5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]carbamoyl]-1H-benzimidazole-2-yl]phenyl]carbamoylethyl]dimethylsulfonium iodide

0.10 g (0.17 mmol) of 1H-2-[3-(3-methylthiopropionylamino)phenyl]benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was dissolved in a mixture of 0.5 ml of 85% formic acid and 0.25 ml of acetic acid, and 0.2 ml of methyl iodide was then added, followed by stirring at room temperature for 3 days under shading. After concentration under reduced pressure, the resulting residue was subjected to gel filtration (Sephadex LH-20, methanol), and then solidified with ether, thereby obtaining 0.10 g (0.13 mmol, 81.2%) of the desired compound in the state of light yellowish white powder.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.48 (s, 1H), 10.35 (s, 1H), 8.58 (s, 1H), 8.25 (s, 1H), 8.02 (s, 1H), 7.86 (m, 2H), 7.71 (m, 3H), 7.55 (t, 1H), 7.36 (d,1H), 3.64-3.48 (m, 8H), 3.03 (t, 2H), 2.97 (s, 6H)

IR (KBr) cm^{-1}: 3248, 1655, 1577, 1497, 1389, 1307

Elemental analysis: ($C_{29}H_{31}Cl_3IN_5O_3S \cdot 2.5H_2O$)			
Calcd.:	C:43.98,	H:4.58,	N:8.84
Found:	C:44.00,	H:4.26,	N:8.62

Example 22 (Compound 2051)

1H-2-[3-[(3-morpholinopropionyl)amino]phenyl]benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide hydrochloride

In DMF-methanol, 0.51 g (1.02 mmols) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.21 ml (1.51 mmols, 1.5 eq.) of triethylamine and a chloroform solution containing 1.53 mmols of 3-morpholinopropionyl chloride {which was prepared from 0.30 g (1.53 mmols) of 3-morpholinopropionic acid and 1 ml of thionyl chloride in chloroform} were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was stirred for 3 hours and then allowed to stand overnight. Since the material did not disappear the next day, the chloroform solution containing 1.53 mmols of 3-morpholinopropionyl chloride was further added. After stirring for 9 hours, the solution was concentrated under reduced pressure, and the resulting residue was subjected to silica gel column chromatography (chloroform/methanol = 8-12%). Next, the eluted fraction was concentrated, and methanol was then added. Afterward, the resulting solid was collected by filtration to obtain 0.18 g (0.28 mmol, 27.3%) of the desired compound in the state of light yellowish white powder. Furthermore, the filtrate was subjected to gel filtration (Sephadex LH-20, methanol) and then solidified with ether, thereby obtaining 0.11 g (0.17 mmol, 16.7%) of the desired compound in the state of white powder (total yield = 44.0%).

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.30 (s, 1H), 10.08 (s, 0.4H), 10.01 (s, 0.6H), 8.57 (s, 0.4H), 8.54 (s, 0.6H), 8.33 (s, 0.6H), 8.12 (s, 0.4H), 7.88-7.47 (m, 7H), 6.77 (d, 2H), 3.74 (s, 8H), 3.60 (m, 4H), 2.67 (m, 2H), 2.55-2.44 (m, 6H)
IR (KBr) cm^{-1}: 3120, 1622, 1591, 1519, 1341, 1183, 1115

Elemental analysis: ($C_{31}H_{34}Cl_2N_6O_3 \cdot HCl \cdot 4H_2O$)			
Calcd.:	C:51.85,	H:6.04,	N:11.70
Found:	C:51.81,	H:6.22,	N:12.12

Example 23 (Compound 2053)

1H-2-[3-[(3-morpholinopropionyl)amino]phenyl]benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)]amino-3-methyl]phenyl]carboxyamide hydrochloride

In a mixed solvent of 10 ml of DMF and 10 ml of methanol was dissolved 0.50 g (1.00 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)]amino-3-methyl]phenyl]carboxyamide, and catalytic hydrogenation was then carried out under a nitrogen atmosphere in the presence of 10% Pd/C as a catalyst to lead the above-mentioned compound to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.59 ml (3.0 mmols) of morpholinoethylcarboxylic acid hydrochloride and 0.62 g (3.00 mmols) of DCC were added in this order. Afterward, the temperature of the solution was returned to room temperature, and the solution was then allowed to stand overnight. After the reaction, the reaction solution was concentrated under reduced pressure, and the resulting brown syrup-like residue was purified through silica gel column chromatography (chloroform/methanol = 20/1-10/1). Next, the resulting fluorescent fraction was taken out, concentrated, and

then sludged with ether/chloroform to obtain 0.25 g (0.38 mmol, 38.0%) of the desired compound in the state of white powder.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.25 (s, 1H), 10.18 (s, 0.5H), 10.13 (s, 0.5H), 8.55 (d, 1H), 3.34 (s, 0.5H), 8. 12 (s, 0.5H), 7.88-7.48 (m, 7H), 7.24 (d, 1H), 3.61-3.50 (m, 8H), 3.64 (t, 4H), 2.67 (t, 2H), 2.54 (t, 2H), 2.43 (bs, 4H), 2.31 (s, 3H)

IR (KBr) cm^{-1}: 3258, 2963, 1648, 1502, 1446, 1314, 1115

Example 24 (Compound 2054)

1H-2-[3-[(3-morpholinopropionyl)amino]phenyl]benzimidazole-5-[N-[3-chloro-4-[N-bis(2-chloroethyl)amino]phenyl]]carboxyamide hydrochloride

In DMF-methanol, 0.30 g (0.56 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.16 ml (1.15 mmols, 2.0 eq.) of triethylamine and a chloroform solution containing 1.13 mmols of 3-morpholinopropionyl chloride {which was prepared from 0.22 g (1.13 mmols) of 3-morpholinopropionic acid and 1 ml of thionyl chloride in chloroform} were added in this order. Afterward, the temperature of the solution was returned to room temperature, followed by stirring. After 3 hours, 0.16 ml (1.15 mmols) of triethylamine and the chloroform solution containing 1.13 mmols of 3-morpholinopropionyl chloride were further added, and the solution was then allowed to stand overnight. After concentration under reduced pressure, the resulting residue was subjected to silica gel column chromatography (chloroform/methanol = 12%). The eluted fraction was further subjected to gel filtration (Sephadex LH-20, methanol), and 4N hydrochloric acid and dioxane were added, followed by concentration. Next, solidification was accomplished with a small amount of ethanolether, thereby obtaining 0.13 g (0.18 mmol, 32.4%) of the desired compound in the state of light yellowish white amorphous powder.

mp: A definite melting point was not present.

NMR (DMSO-d_6) δ: 10.80 (s, 1H), 10.61 (s, 1H), 8.62 (s, 1H), 8.37 (s, 1H), 8.04 (m, 3H), 7.87-7.73 (m, 3H), 7.64 (t, 1H), 7.37 (d, 1H), 3.87-3.79 (m, 4H), 3.62 (t, 4H), 3.51 (t, 4H), 3.44 (m, 4H), 3.06 (m, 4H)

IR (KBr) cm^{-1}: 3214, 1671, 1576, 1497, 1394, 1307, 1128, 1087

Elemental analysis: ($C_{31}H_{33}Cl_3N_6O_3 \cdot HCl \cdot 6.5H_2O$)			
Calcd.:	C:46.69,	H:5.94,	N:10.54
Found:	C:46.53,	H:5.95,	N:10.45

Example 25 (Compound 2061)

1H-2-[3-(3-pyridylacetylamino)phenyl]benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]carboxyamide

In DMF-methanol, 0.40 g (0.80 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.21 g (1.21 mmols, 1.5 eq.) of 3-pyridylacetic acid hydrochloride and 0.23 g (1.20 mmols, 1.5 eq) of EDCI were added in this order. Afterward, the temperature of the solution was returned to room temperature, followed by stirring for 2.5 hours. After concentration under reduced pressure, the resulting residue was subjected to silica gel column chromatography (chloroform/methanol = 8%), and then solidified with ether. Since some impurities were contained in the powder, a separating operation was carried out by the use of chloroform and dilute ammonia water to precipitate insolubles. Next, the insolubles were collected by filtration to obtain 49 mg (0.083 mmol, 10.4%) of the desired compound in the state of light brown solid. Furthermore, the resulting chloroform layer was concentrated, and then solidified with ether, thereby obtaining 65 mg (0.10 mmol, 12.5%) of the desired hydrochloride in the state of light brown powder.

mp: A definite melting point was not present.

Free form: NMR (DMSO-d_6) δ: 10.47 (s, 1H), 10.00 (s, 0.5H), 8.57 (s, 1H), 8.55 (d, 1H), 8.48 (d, 1H), 8.32 (s, 0.5H), 8.10 (s, 0.5H), 7.87-7.49 (m, 8H), 7.38 (t, 1H), 6.77 (d, 2H), 3.77 (s, 2H), 3.73 (s, 8H)

Hydrochloride: NMR ($DMSO-d_6$) δ: 10.52 (s, 1H), 10.03 (s, 1H), 8.62 (s, 1H), 8.54 (m, 2H), 8.22 (s, 1H), 7.91-7.84 (m, 3H), 7.72-7.62 (m, 4H), 7.55-7.45 (m, 2H), 6.77 (d, 2H), 3.81 (s, 2H), 3.73 (s, 8H)

IR (KBr) cm^{-1}: 3070, 1617, 1518, 1328, 1184, 1141

Elemental analysis (free form): ($C_{31}H_{28}Cl_2N_6O_2 \cdot 2.5H_2O$)			
Calcd.:	C:58.86,	H:5.26,	N:13.29
Found:	C:58.86,	H:5.44,	N:13.28

Example 26 (Compound 2063)

1H-2-[3-(3-pyridylacetylamino)phenyl]benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide

In 10 ml of a mixed solvent of DMF:methanol = 1:1, 0.40 g (0.80 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.4 g (2.3 mmols, 3 eq.) of 3-pyridylacetic acid hydrochloride and 0.45 g (2.4 mmols, 3 eq) of EDCI were added in this order, followed by stirring for 30 minutes as it was. After stirred at room temperature for further 3 hours, the solution was allowed to stand overnight. After the solvents were distilled off under reduced pressure, the resulting residue was purified through silica gel column chromatography (chloroform/methanol = 95/5). The solvents were distilled off, and the residue was then dissolved in methanol. Next, isopropyl ether was added, so that precipitation occurred again, to obtain 153 mg (yield = 33%) of white powder.

NMR ($DMSO-d_6$) δ: 10.48 (s, 1H), 10.18 (bs, 0.5H), 10.13 (bs, 0.5H), 8.61-7.36 (m, 13H), 7.23 (d, 1H), 3.77 (s, 2H), 3.59 (t, 4H), 3.36 (t, 4H), 2.31 (s, 3H)

IR (KBr) cm^{-1}: 3348, 1665, 1504, 1308, 1263, 884

Example 27 (Compound 2064)

1H-2-[3-(3-pyridylacetylamino)phenyl]benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide

In DMF-methanol, 0.40 g (0.75 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[3-chloro-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.21 g (1.21 mmols, 1.6 eq.) of 3-pyridylacetic acid and 0.22 g (1.15 mmols, 1.5 eq) of EDCI were added in this order. Afterward, the temperature of the solution was returned to room temperature, followed by stirring for 3.5 hours. After concentration under reduced pressure, the resulting residue was subjected to a separating operation by the use of chloroform and water to precipitate gel-like insolubles. Next, the resulting aqueous layer was adjusted to a pH of about 11 with an aqueous sodium hydroxide solution and then extracted with chloroform twice. The obtained chloroform layers were joined to each other, dried over anhydrous sodium sulfate, and then concentrated under reduced pressure. Afterward, the residue was solidified with ether, thereby obtaining 96 mg (0.15 mmol, 20.6%) of the desired hydrochloride in the state of light brown powder.

mp: A definite melting point was not present.

NMR ($DMSO-d_6$) δ: 10.47 (s, 1H), 10.37 (s, 0.4H), 10.31 (s, 0.6H), 8.57 (s, 1H), 8.48 (d, 1H), 8.34 (s, 0.6H), 8.11 (s, 0.4H), 8.02 (s, 1H), 7.87-7.61 (m, 7H), 7.52 (t, 1H), 7.40-7.35 (m, 2H), 3.77 (s, 2H), 3.61 (t, 4H), 3.50 (t, 4H)

IR (KBr) cm^{-1}: 3191, 1631, 1582, 1498, 1390, 1309

Example 28 (Compound 2073)

1H-2-[3-(4-pyridylacetylamino)phenyl]benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide

In 10 ml of a mixed solvent of DMF:methanol = 1:1, 0.40 g (0.80 mmol) of 1H-2-(3-nitrophenyl)benzimidazole-5-[N-[3-methyl-4-[N,N-bis(2-chloroethyl)amino]phenyl]]carboxyamide was subjected to catalytic hydrogenation using 10% Pd/C as a catalyst to lead it to a corresponding amino compound. Next, the DMF solution of this amino compound was stirred under a nitrogen gas stream while cooled on ice, and 0.4 g (2.3 mmols, 3 eq.) of 4-pyridylacetic acid hydrochloride and 0.45 g (2.4 mmols, 3 eq) of EDCI were added in this order, followed by stirring for 30 minutes as it was. After stirred at room temperature for additional 6 hours, the solution was allowed to stand overnight. After the solvents were distilled off under reduced pressure, the resulting residue was purified through silica gel column chromatography (chloroform/methanol = 6/1). The solvents were distilled off, and the residue was further purified through silica gel column chromatography (chloroform/methanol = 8/1). The solvents were distilled off, and the residue was then dissolved in methanol. Next, isopropyl ether was added, so that precipitation occurred again, to obtain 50 mg (yield = 11%) of light yellow powder.

NMR (DMSO-d_6) δ: 10.51 (s, 1H), 10.18 (bs, 0.5H), 10.13 (bs, 0.5H), 8.61 (s, 1H), 8.55 (d, 2H), 8.33 (s, 0.5H), 8.11 (s, 0.5H), 7.89-7.49 (m, 7H), 7.39 (d, 2H), 7.23 (d, 1H), 3.77 (s, 2H), 3.57 (t, 4H), 3.36 (t, 4H), 2.31 (s, 3H)

IR (KBr) cm^{-1}: 3422, 1647, 1508, 1318, 1239, 829

Formulation Example 1

Compound No. 2053 as an active ingredient	30 g
Lactose	68 g
Crystalline cellulose	20 g
Magnesium stearate	2 g

The components described above are mixed in the above composition and the resulting mixture was formulated into core tablets by a tableting machine. Each of the core tablets weighed 120 mg containing 30 mg of Compound No. 2053 and had a diameter of 7 mm.

Talc was then sprinkled on each core tablet and the surface having talc was then coated with varnish to form an undercoat. Additional varnish coating was repeated so as to obtain tablets suitable for the internal uses. Color coating was further conducted. After drying, the tablets having the color coats were waxed and polished into tablets of uniform gloss.

Formulation Example 2

As an active ingredient, 1 g of Compound No. 2053 was weighed and dissolved in 1,000 ml of sterilized propylene glycol. The resting solution was poured and enclosed in ampoules so as to obtain injections in ampoules, each of which contained 5 ml of the solution.

Test Example 1

Investigation was made on the linkage of each compound to DNA. The test was carried out by comparing a Tm value in the case that the compound was added to the DNA solution with a Tm value in the case that no compound was added thereto.

That is to say, poly d(A-T)-d(A-T) was used as the DNA. This DNA was dissolved in a buffer solution and the compound was further added thereto, and the Tm value was then measured. On the other hand, the Tm of the DNA alone was measured, and a difference (ΔTm) was then calculated. For the measurement, a U-3200 model spectrophotometer made by Hitachi, Ltd. was used, and for the control of temperature, SPR-10 model made by Hitachi, Ltd. was used.

The ΔTm of Compound 2001 was obtained, and it was 14°C.

Test Example 2

The anticancer activity of each compound will be described. Table 2 shows the anti-tumor activity of the typical compounds. This test was carried out by measuring an in vitro inhibitory activity on tumor cell growth. That is to say, the B16 melanoma cells of a mouse were planted on a culture plate having 96 cells, and a compound was then added after one day and the cells were cultured at 37°C for 3 days in 5% CO_2. Next, a compound concentration (IC_{50}) necessary to accomplish a 50% growth inhibitory effect was determined in accordance with a procedure described in Cancer. Res., Vol. 48, p. 589-601 (1988). The unit of the drug concentration was μg/ml. The result of distamycin is also simultaneously shown as a comparative example.

Table 2

Anti-tumor activity	
Compound No.	IC_{50}(μg/ml)
2	0.58
29	3.05
47	0.38
1001	0.36
1010	3.19
2001	0.82
2004	0.53
2006	0.65
2011	0.97
2014	1.18
2016	2.08
2031	1.56
2041	0.37
2044	0.72
2046	0.39
2051	0.49
2053	0.33
2054	0.41
2063	3.48
2073	1.19
Distamycin	36

Test Example 3

A cell floating solution having 1×10^7 mouse colon cancer cells of Colon 26 per ml of HBSS (Hanks' Balanced Salt Solution) was prepared. Next, 0.1 ml of this cell floating solution was implanted in the side abdomen hypodermis of a female CDF1 mouse (0 day). On the day after the tumor implantation (1st day), the weight of the mouse was measured, and a solution of a compound (a 5% glucose solution containing 5% Tween 80) was administered into the tail vein of the mouse. On the 15th day, the tumor was extracted, and its weight was then measured.

An average tumor weight ratio of each experimental sample was calculated as a T/C value on the assumption that the average tumor weight ratio of a control to which any compund was not administered was 100%.

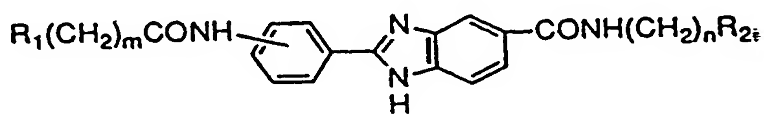
Table 3 shows the results of the test. The T/C values correspond to compound numbers, respectively, and each value in the parentheses represents the concentration of the drug at the time when its T/C value was obtained.

Table 3

Compound No.	T/C (%)	Dose (mg/kg)
2001	13	30
2006	21	10
Adriamycin	29	20

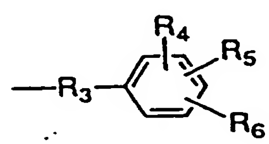
Claims

1. A compound represented by the following formula (1) or its pharmacologically acceptable salt:



(1)

wherein each of m and n is independently an integer of from 0 to 5; each of R_1 and R_2 is independently a hydrogen atom, a halogen atom, an alkylthio group having 1 to 8 carbon atoms, an amino group which may be substituted, an ammonium group which may be substituted, a sulfonium group which may be substituted, a phenyl group which may be substituted, a hetero-five-membered ring group which may be substituted, a hetero-six-membered ring group which may be substituted, an amidino group, a guanidino group, an amino acid residue or a group represented by the formula (2)



(2)

wherein R_3 is a direct bond or an oxygen atom {when R_3 is an oxygen atom, m or n of $(CH_2)_m$ or $(CH_2)_n$ to which R_3 bonds is not 0}; R_4 is a hydrogen atom, an alkyl group having 1 to 8 carbon atoms, an alkoxy group having 1 to 8 carbon atoms, a halogen atom, a trifluoromethyl group, a cyano group, an amidino group, a carboxyl group or -COR_7 wherein R_7 is an alkylamino group having 1 to 8 carbon atoms which may be substituted by a substituted amino group, an amino group which may be substituted by a phenyl group which may be substituted, or a benzylamino group which may be substituted; R_5 is a hydrogen atom, an alkyl group having 1 to 8 carbon atoms, an alkoxy group having 1 to 8 carbon atoms or a halogen atom; R_6 is a -$(CH_2)_pN(R_8)_2$ or -$(CH_2)_pNR_8R_9$ wherein p is an integer of from 0 to 5; R_8 is -CH_2CH_2W wherein W is a halogen atom, a hydroxyl group, a mesyloxy group or a tosyloxy group or -$OCOR_7$ wherein R_7 is as defined above; R_9 is an alkyl group having 1 to 5 carbon atoms or a mesyl group;

the phenyl group having a $R_1(CH_2)_mCONH$ group in formula (1) can be substituted by the $R_1(CH_2)_mCONH$ group at any position.
position of the phenyl group.

2. The compound or its pharmacologically acceptable salt according to claim 1 wherein R_1 and/or R_2 is an alkylthio group having 1 to 4 carbon atoms.

3. The compound or its pharmacologically acceptable salt according to claim 1 wherein the phenyl group having a $R_1(CH_2)_mCONH$ group in formula (1) is substituted by the $R_1(CH_2)_mCONH$ group at the 3- or 4-position of the phenyl group.

4. The compound or its pharmacologically acceptable salt according to Claim 1 or 3 wherein R_1 is a halogen atom or a group represented by formula (2).

5. The compound or its pharmacologically acceptable salt according to any one of Claims 1, 3 or 4 wherein R_2 is a halogen atom or a group represented by formula (2).

6. The compound or its pharmacologically acceptable salt according to Claim 4 wherein R_2 is an amino group which may be substituted, a guanidino group or an amidino group.

7. The compound or its pharmacologically acceptable salt according to Claim 5 wherein R_1 is an amino group which may be substituted, a guanidino group or an amidino group.

8. A pharmaceutical composition comprising as an active ingredient, a compound or its pharmacologically acceptable salt described in any one of Claims 1 to 7.

9. A pharmaceutical composition according to claim 8 which is an anticancer composition.

10. A pharmaceutical composition according to claim 8 which is an antimicrobial composition.

11. A pharmaceutical composition according to claim 8 which is an antiviral composition.

12. Use of a compound or its pharmacologically acceptable salt described in any one of Claims 1 to 7 for the preparation of an anticancer agent, an antimicrobial agent or an antiviral agent.

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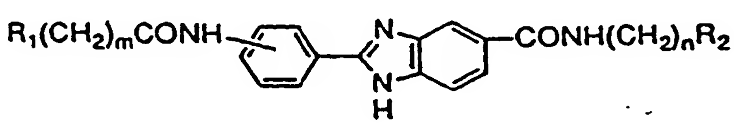
(54) Phenylbenzimidazole derivatives

(57) An anticancer agent, an antiviral agent or an antimicrobial agent which contains, as an active ingredient for acting on DNA, a compound presented by the following formula (1) or its pharmacologically acceptable salt:



(1)

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EUROPEAN SEARCH REPORT

Application Number
EP 95 12 0576

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	ANTIVIRAL RESEARCH, vol. 22, no. 2-3, 1993, pages 107-19, XP000196600 R. OESTERLE ET AL.: "Chemical modifications of aminonaphthalenesulfonic acid derivatives increase effectivity and specificity of reverse transcriptase inhibition and change mode of action of reverse transcriptase and DNA polymerase alpha inhibition" * page 107, summary; page 111, table 1; page 114, table 2; page 115, compound NF415 * ---	1,3,8,11	C07D235/18 A61K31/415
X	KHIM.-FARM. ZHURNAL, vol. 22, no. 6, 1988, pages 697-9, XP000196617 I.V. SKLYAROVA ET AL.: "Synthesis and biological activity of 5(6)-amido- and 5(6)-amino-2-arylbenzoimidazole derivatives" [original title in Russian] * page 697, compound III * & Chem. Abs. 110:192707 ---	1,3	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C07D
A,D	EP-A-0 246 868 (FARMITALIA CARLO ERBA S.P.A.) * claims 1,6-9 * ---	1,8,9, 11,12	
A,D	WO-A-93 13739 (A. MENARINI INDUSTRIE FARMACEUTICHE RIUNITE S.R.L.) * claims 1,23-26 * ---	1,8,9,11	
A,D	US-A-5 273 991 (M.N.F.LEE) * abstract; claims 1,20,41 * --- -/--	1,8,9	

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	11 December 1996	Hass, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
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& : member of the same patent family, corresponding document

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EUROPEAN SEARCH REPORT

Application Number
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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A,D	JOURNAL OF MEDICINAL CHEMISTRY, vol. 32, 1989, pages 774-8, XP000608784 F. M. ARCAMONE ET AL.: "Synthesis, DNA-Binding Properties, and Antitumor Activity of Novel Distamycin Derivatives"	1	
A	EP-A-0 194 529 (GÖDECKE AG) * claim 1 *	1,9	
A	EP-A-0 148 431 (DR. KARL THOMAE GMBH) * claim 1 *	1	
A	EP-A-0 209 707 (DR. KARL THOMAE GMBH) * claim 1 *	1	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
BERLIN	11 December 1996	Hass, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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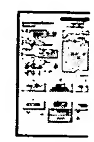
Ema

Title: **EP1077700A1: BENZIMIDAZOLE DERIVATIVES AS MODULATORS O** [German][French]

Derwent Title: Use of benzimidazole derivatives for the treatment of allergy and/or asthma or any disease where IgE (Immunoglobulin E) is pathogenic [Derwent Record]

Country: **EP** European Patent Office (EPO)

Kind: **A1** Publ. of Application with search report i (See also: EP1077700B1, WO09961019)



Inventor: **SIRCAR, Jagadish, Avanir Pharmaceuticals**; **RICHARDS, Mark, L., Avanir Pharmaceuticals**; **CAMPBELL, Michael, G., Avanir Pharmaceuticals**; **MAJOR, Michael, W., Avanir Pharmaceuticals**;

Assignee: **Avanir Pharmaceuticals**
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Published / Filed: **2001-02-28** / 1999-05-21

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ECLA Code: **A61K31/00**; A61K31/415+M; A61K31/4184; A61K31/4184+M; A61K31/4545; A61K31/4545+M; A61K45/06; A61K45/06+M;

Priority Number: 1998-05-22 **US1998000086494P**

Attorney, Agent or Firm: **W.P. Thompson &, Co.** ;

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Designated Country: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI

Family: Show 78 known family members

Description: Collapse

[From equivalent EP1077700B1] **Background of the Invention**

This invention relates to small molecule inhibitors of the IgE response to allergens that are useful in the treatment of allergy and/or asthma or any diseases where IgE is pathogenic.

An estimated 10 million persons in the United States have asthma, about 5% of the population. The estimated cost of asthma in the United States exceeds $6 billion. About 25% of patients with asthma who seek emergency care require hospitalization, and the largest single direct medical expenditure for asthma has been

inpatient hospital services (emergency care), at a cost of greater than $1.6 billion. The cost for prescription medications, which increased 54% between 1985 and 1990, was close behind at $1.1 billion (Kelly, *Pharmacotherapy* 12:13S-21S (1997)).

According to the National Ambulatory Medical Care Survey, asthma accounts for 1% of all ambulatory care visits, and the disease continues to be a significant cause of missed school days in children. Despite improved understanding of the disease process and better drugs, asthma morbidity and mortality continue to rise in this country and worldwide (U.S. Department of Health and Human Services; 1991, publication no. 91-3042). Thus, asthma constitutes a significant public health problem.

The pathophysiologic processes that attend the onset of an asthmatic episode can be broken down into essentially two phases, both marked by bronchoconstriction, that causes wheezing, chest tightness, and dyspnea. The first, early phase asthmatic response is triggered by allergens, irritants, or exercise. Allergens cross-link immunoglobulin E (IgE) molecules bound to receptors on mast cells, causing them to release a number of pre-formed inflammatory mediators, including histamine. Additional triggers include the osmotic changes in airway tissues following exercise or the inhalation of cold, dry air. The second, late phase response that follows is characterized by infiltration of activated eosinophils and other inflammatory cells into airway tissues, epithelial desquamonon, and by the presence of highly viscous mucus Within the airways. The damage caused by this inflammatory response leaves the airways "primed" or sensitized, such that smaller triggers are required to elicit subsequent asthma symptoms.

A number of drugs are available for the palliative treatment of asthma; however, their efficacies vary markedly. Short-acting β_2-adrenergic agonists, terbutaline and albuterol, long the mainstay of asthma treatment, act primarily during the early phase as bronchodilators. The newer long-acting β_2-agonists, salmeterol and formoterol, may reduce the bronchoconstrictive component of the late response. However, because the β_2-agonists do not possess significant antiinflammatory activity, they have no effect on bronchial hyperreactivity.

Numerous other drugs target specific aspects of the early or late asthmatic responses. For example, antihistamines, like loratadine, inhibit early histamine-mediated inflammatory responses. Some of the newer antihistamines, such as azelastine and ketotifen, may have both antiinflammatory and weak bronchodilatory effects, but they currently do not have any established efficacy in asthma treatment. Phosphodiesterase inhibitors, like theophylline/xanthines, may attenuate late inflammatory responses, but there is no evidence that these compounds decrease bronchial hyperreactivity. Anticholinergics, like ipratopium bromide, which are used in cases of acute asthma to inhibit severe bronchoconstriction, have no effect on early or late phase inflammation, no effect on bronchial hyperreactivity, and therefore, essentially no role in chronic therapy.

The corticosteroid drugs, like budesonide, are the most potent antiinflammatory agents. Inflammatory mediator release inhibitors, like cromolyn and nedocromil, act by stabilizing mast cells and thereby inhibiting the late phase inflammatory response to allergen. Thus, cromolyn and nedocromil, as well as the corticosteroids, all reduce bronchial hyperreactivity by minimizing the sensitizing effect of inflammatory damage to the airways. Unfortunately, these antiinflammatory agents do not produce bronchodilation.

Several new agents are currently being developed that inhibit specific aspects of asthmatic inflammation. For instance, leukotriene receptor antagonists (ICI-204, 219, accolate), specifically inhibit leukotriene-mediated actions. The leukotrienes have been implicated in the production of both airway inflammation and bronchoconstriction.

Thus, while numerous drugs are currently available for the treatment of asthma, these compounds are primarily palliative and/or have significant side effects. Consequently, new therapeutic approaches which target the underlying cause rather than the cascade of symptoms would be highly desirable. Asthma and allergy share a common dependence on IgE-mediated events. Indeed, it is known that excess IgE production is the underlying cause of allergies in general and allergic asthma in particular (Duplantier and Cheng, *Ann. Rep. Med. Chem.* 29:73-81 (1994)). Thus, compounds that lower IgE levels may be effective in treating the underlying cause of asthma and allergy.

None of the current therapies eliminate the excess circulating IgE. The hypothesis that lowering plasma IgE may reduce the allergic response, was confirmed by recent clinical results with chimeric anti-IgE antibody, CGP-51901, and recombinant humanized monoclonal antibody, rhuMAB-E25. Indeed, three companies, Tanox Biosystems, Inc., Genentech Inc. and Novartis AG are collaborating in the development of a humanized anti-IgE antibody (BioWorld® Today, February 26, 1997, p. 2) which will treat allergy and asthma by neutralizing excess IgE. Tanox has already successfully tested the anti-IgE antibody, CGP-51901, which reduced the severity and duration of nasal symptoms of allergic rhinitis in a 155-patient Phase II trial (Scrip #2080, Nov 24, 1995, p.26). Genentech recently disclosed positive results from a 536 patient phase-II/III trials of its recombinant humanized monoclonal antibody, rhuMAB-E25 (BioWorld® Today, November 10, 1998, p. 1). The antibody, rhuMAB-E25, administered by injection (highest dose 300 mg every 2 to 4 weeks as needed) provided a 50% reduction in the number of days a patient required additional "rescue" medicines (antihistimines and decongestants), compared to placebo. An NDA filing for this product is projected to be in the year 2000. The positive results from anti-IgE antibody trials suggest that therapeutic strategies aimed at IgE down-regulation may be effective.

Summary of the Invention

The present invention discloses a family of related compounds for use in the treatment of a condition associated with an excess IgE level. The benzimidazole inhibitors of IgE in accordance with the present invention are represented by the generic formula: X and Y are independently selected from the group consisting of H, alkyl, alkoxy, aryl, substituted aryl, hydroxy, halogen, amino, alkylamino, nitro, cyano, CF_3, OCF_3, $CONH_2$, CONHR and $NHCOR_1$. R is selected from the group consisting of H, CH_3, C_2H_5, C_3H_7, C_4H_9, CH_2Ph, and $CH_2C_6H_4$-F(p-). R_1 and R_2 are independently selected from the group consisting of H, aryl, substituted aryl, cycloaryl substituted cycloaryl, multi-ring cycloaryl, benzyl, substituted benzyl and the like. Substitutions are alkyl, aryl, CF_3, CH_3, OCH_3, OH, CN, COOR, COOH and the like.

In accordance with another aspect of the invention, there is disclosed a composition for use in the treatment of an allergic condition comprising the diacyl benzimidazole inhibitor of IgE disclosed above and at least one additional active ingredient,

phosphodiesterase inhibitor, an anticholinergic agent, a corticosteroid, an inflammatory mediator release inhibitor or a leukotriene receptor antagonist.

The compound is preferably administered at a dose of about 0.01 mg to about 100 mg per kg body weight per day in divided doses of said compound for at least two consecutive days at regular periodic intervals.

Other variations within the scope of the present invention may be more fully understood with reference to the following detailed description.

Detailed Description of the Preferred Embodiment

The present invention is directed to small molecule inhibitors of IgE (synthesis and/or release) which are useful in the treatment of allergy and/or asthma or any diseases where IgE is pathogenic. The particular compounds disclosed herein were identified by their ability to suppress IgE levels in both *ex vivo* and *in vivo* assays. Development and optimization of clinical treatment regimens can be monitored by those of skill in the art by reference to the *ex vivo* and *in vivo* assays described below.

***Ex Vivo* Assay**

This assay begins with *in vivo* antigen priming and measures secondary antibody responses *in vitro*. The basic protocol was documented and optimized for a range of parameters including: antigen dose for priming and time span following priming, number of cells cultured *in vitro,* antigen concentrations for eliciting secondary IgE (and other Ig's) response *in vitro*, fetal bovine serum (FBS) batch that will permit optimal IgE response *in vitro,* the importance of primed CD4+ T cells and hapten-specific B cells, and specificity of the ELISA assay for IgE (Marcelletti and Katz, *Cellular Immunology* 135:471-489 (1991); incorporated herein by reference).

The actual protocol utilized for this project was adapted for a more high throughput analyses. BALB/cByj mice were immunized i.p. with 10 µg DNP-KLH adsorbed onto 4 mg alum and sacrificed after 15 days. Spleens were excised and homogenized in a tissue grinder, washed twice, and maintained in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin and 0.0005% 2-mercaptoethanol. Spleen cell cultures were established (2-3 million cells/ml, 0.2 ml/well in quadruplicate, 96-well plates) in the presence or absence of DNP-KLH (10 ng/ml). Test compounds (2 µg/ml and 50 ng/ml) were added to the spleen cell cultures containing antigen and incubated at 37 ° C for 8 days in an atmosphere of 10% CO_2.

Culture supernatants were collected after 8 days and Ig's were measured by a modification of the specific isotype-selective ELISA assay described by Marcelletti and Katz (*Supra*). The assay was modified to facilitate high throughput. ELISA plates were prepared by coating with DNP-KLH overnight. After blocking with bovine serum albumin (BSA), an aliquot of each culture supernatant was diluted (1:4 in phosphate buffered saline (PBS) with BSA, sodium azide and Tween 20), added to the ELISA plates, and incubated overnight in a humidified box at 4° C. IgE levels were quantitated following successive incubations with biotinylated-goat antimouse IgE (b-GAME), AP-streptavidin and substrate.

Antigen-specific IgG1 was measured similarly, except that culture supernatants were diluted 200-fold and biotinylated-goat antimouse IgG1 (b-GAMG1) was substituted for b-GAME. IgG2a was measured in ELISA plates that were coated with DNP-KLH following

combined in a pharmaceutically acceptable diluent. The additional active ingredients may be selected from the group consisting of short-acting β_2-adrenergic agonists, like terbutaline and albuterol, long-acting β_2-adrenergic agonists, like salmeterol and formoterol, antihistamines, like loratadine, azelastine and ketotifen, phosphodiesterase inhibitors, anticholinergic agents, corticosteroids, inflammatory mediator release inhibitors and leukotriene receptor antagonists.

In accordance with another aspect of the invention, there is disclosed a family of symmetric and asymmetric diacyl and monoacyl benzimidazole compounds for use in the treatment of an allergic condition comprising the following species:

In accordance with another aspect of the present invention, there is disclosed a method for the preparation of a medicament for treatment of a condition associated with an excess IgE level. The compound has the formula: X and Y are independently selected from the group consisting of H, alkyl, alkoxy, aryl, substituted aryl, hydroxy, halogen, amino, alkylamino, nitro, cyano, CF_3, OCF_3, $CONH_2$, CONHR and $NHCOR_1$. R is selected from the group consisting of H, CH_3, C_2H_5, C_3H_7, C_4H_9, CH_2Ph, and $CH_2C_6H_4$-F(p-). R_1 and R_2 are independently selected from the group consisting of H, aryl, substituted aryl, cycloaryl substituted cycloaryl, multi-ring cycloaryl, benzyl, substituted benzyl and the like. Substitutions are alkyl, aryl, CF_3, CH_3, OCH_3, OH, CN, COOP, COOH and the like.

In accordance with another aspect of the present invention, there is disclosed a method of treating a mammal having a condition associated with an excess IgE level. The method comprises administering to the mammal an amount of a compound sufficient to reduced IgE levels in the mammal. The compound has the formula: X and Y are independently selected from the group consisting of H, alkyl, alkoxy, aryl, substituted aryl, hydroxy, halogen, amino, alkylamino, nitro, cyano, CF_3, OCF_3, $CONH_2$, CONHR and $NHCOR_1$. R is selected from the group consisting of H, CH_3, C_2H_5, C_3H_7, C_4H_9, CH_2Ph, and $CH_2C_6H_4$-F(p-). R_1 and R_2 are independently selected from the group consisting of H, aryl, substituted aryl, cycloaryl substituted cycloaryl, multi-ring cycloaryl, benzyl, substituted benzyl, alkyl, cycloalkyl substituted cycloalkyl, multi-ring cycloalkyl, fused-ring aliphatic, cyclopropyl, substituted cyclopropyl, cyclobutyl, substituted cyclobutyl, cyclopentyl, substituted cyclopentyl, cyclohexyl, substituted cyclohexyl, cycloheptyl, substituted cycloheptyl, bicycloheptyl, bicyclooctyl, bicyclononyl, substituted bicycloalknyl, adamantyl, substituted adamantyl and the like, wherein at least one of R1 and R2 are aromatic groups. Substitutions are alkyl, aryl, CF_3, CH_3, OCH_3, OH, CN, COOR, COOH and the like.

In a variation of the above-disclosed method, at least one additional active ingredient may be administered in conjunction with the administration of the compound. The additional active ingredient may be combined with said compound in a pharmaceutically acceptable diluent and co-administered to the mammal. The additional active ingredient may be a short-acting β_2-adrenergic agonist selected from the group consisting of terbutaline and albuterol. In a variation, the additional active ingredient may be a long-acting β_2-adrenergic agonist selected from the group consisting of salmeterol and formoterol or an antihistamine selected from the group consisting of loratadine, azelastine and ketotifen. In another variation, the additional active ingredient may be a

a 1:20 dilution of culture supernatants and incubation with biotinylated-goat antimouse IgG2a (b-GAMG2a). Quantitation of each isotype was determined by comparison to a standard curve. The level of detectability of all antibody was about 200-400 pg/ml and there was less than 0.001% cross-reactivity with any other Ig isotype in the ELISA for IgE.

***In Vivo* Assay**

Compounds found to be active in the *ex vivo* assay (above) were further tested for their activity in suppressing IgE responses *in vivo*. Mice receiving low-dose radiation prior to immunization with a carrier exhibited an enhanced IgE response to sensitization with antigen 7 days later. Administration of the test compounds immediately prior to and after antigen sensitization, measured the ability of that drug to suppress the IgE response. The levels of IgE, IgG1 and IgG2a in serum were compared.

Female BALB/cByj mice were irradiated with 250 rads 7 hours after initiation of the daily light cycle. Two hours later, the mice were immunized i.p. with 2 μg of KLH in 4 mg alum. Two to seven consecutive days of drug injections were initiated 6 days later on either a once or twice daily basis. Typically, i.p. injections and oral gavages were administered as suspensions (150 μl/injection) in saline with 10% ethanol and 0.25% methylcellulose. Each treatment group was composed of 5-6 mice. On the second day of drug administration, 2 μg of DNP-KLH was administered i.p. in 4 mg alum, immediately following the morning injection of drug. Mice were bled 7-21 days following DNP-KLH challenge.

Antigen-specific IgE, IgG1 and IgG2a antibodies were measured by ELISA. Periorbital bleeds were centrifuged at 14,000 rpm for 10 min, the supernatants were diluted 5-fold in saline, and centrifuged again. Antibody concentrations of each bleed were determined by ELISA of four dilutions (in triplicate) and compared to a standard curve: anti-DNP IgE (1:100 to 1:800), anti-DNP IgG2a (1:100 to 1:800), and anti-DNP IgG1 (1:1600 to 1:12800).

Diacyl Benzimidazole Inhibitors of IgE

Several species embraced by the following generic formula were synthesized and evaluated for their effectiveness in down-regulating IgE in the *ex vivo* and *in vivo* assays.

X and Y are independently selected from the group consisting of H, alkyl, alkoxy, aryl, substituted aryl, hydroxy, halogen, amino, alkylamino, nitro, cyano, CF_3, OCF_3, $CONH_2$, CONHR and $NHCOR_1$. R is selected from the group consisting of H, CH_3, C_2H_5, C_3H_7, C_4H_9, CH_2Ph, and $CH_2C_6H_4$-F(p-). R_1 and R_2 are independently selected from the group consisting of H, aryl, substituted aryl, cycloaryl substituted cycloaryl, multi-ring cycloaryl, benzyl, substituted benzyl, alkyl, cycloalkyl substituted cycloalkyl, multi-ring cycloalkyl, fused-ring aliphatic, cyclopropyl, substituted cyclopropyl, cyclobutyl, substituted cyclobutyl, cyclopentyl, substituted cyclopentyl, cyclohexyl, substituted cyclohexyl, cycloheptyl, substituted cycloheptyl, bicycloheptyl, bicyclooctyl, bicyclononyl, substituted bicycloalknyl, adamantyl, substituted adamantyl and the like, wherein at least one of R1 and R2 are aromatic groups. Substitutions are alkyl, aryl, CF_3, CH_3, OCH_3, OH, CN, COOR, COOH and the like.

Synthesis of the Combinatorial Library

The diacyl benzimidazole compounds of the present invention were prepared using the following synthesis reactions, wherein the

desired acid chlorides are selected from the R1 and R2 groups provided in the Table.

Synthesis of 3: 4-Nitro-1,2-phenylenediamine (10 g, 65.3 mmol) and 4-aminobenzoic acid (8.95 g, 65.3 mmol) were taken in a round bottomed flask and phosphorus oxychloride (95 ml) was added slowly. The reaction mixture was allowed to stir under reflux conditions. After 18 h, the reaction was allowed to cool and then poured slowly into an ice water mixture in an Erlenmeyer flask with vigorous stirring. Greenish yellow precipitate fell out which was then filtered and washed with copious amounts of water. The residue was then dried to obtain 16.9 g of crude desired product. Mass spectrum analysis (positive ion) indicated presence of 3.

Synthesis of 4: Benzimidazole 3 (800 mg, 3.14 mmol) was dissolved in dry pyridine (5 ml) in a scintillation vial and the desired acid chlorides (1.1 eq) were added slowly. The reactions were carried out in an oven at 60C. After 16h, the reaction was cooled to RT and DI water was added. Precipitation took place, which was filtered off, washed with water and air dried. The aqueous layer was extracted with EtOAc (6 x 50 ml), dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo* to result in a colored solid. By positive ion MS the desired monoacylated product was found to be present in the initial precipitate as well as in the organic layer. Hence the solid residues obtained were combined and used as such for the reduction step.

Reduction of 4: Crude monoacylated nitro benzimidazole 4 (1.22 g, 3.40 mmol) was dissolved in MeOH (20 ml) and minimum amount of THF was added for complete dissolution to occur. Catalytic amount of 10% Pd on C was added and the solution was degassed and allowed to stir at 3.4 atm pressure under H_2 atmosphere for 4 h. Upon completion of reaction as observed via TLC, the reaction mixture was filtered through celite and the solvent was removed under reduced pressure to afford 979 mg of crude residue.

General Organic Analyses

HPLC/MS data was obtained using a Gilson semi-prep HPLC with a Gilson 170 Diode Array UV detector and PE Sciex API 100LC MS based detector. A Waters 600E with a Waters 490E UV detector was also used for recording HPLC data. The compounds were eluted with a gradient of CH_3CN (with 0.0035% TFA) and H_2O (with 0.01% TFA). Both HPLC instruments used Advantage C18 60A 5µ 50mm x 4.6mm columns from Thomson Instrument Company. Mass spectra were obtained by direct injection and electrospray ionization on a PE Sciex API 100LC MS based detector. Thin layer chromatography was performed using Merck 60F-254 aluminum backed precoated plates. Flash chromatography was carried out on Merck silica gel 60 (230-400 mesh) purchased from EM Scientific.

Syntheses of Symmetrical Diamides

The symmetrical diacyl benzimidazole compounds of the present invention were generally prepared from 2-(4-aminophenyl)-5-aminobenzimidazole, which was obtained by reduction of 2-(4-nitrophenyl)-6-nitrobenzimidazole. The dinitro benzimidazole was prepared as follows: a mixture of 4-nitrophenylenediamine (6.4g, 41.83 mmol) and 4-nitrobenzoic acid (7.86 g, 47 mmol) was dissolved in $POCl_3$ (250 ml) and heated to reflux for 2 h. The reaction mixture was cooled, poured on to ice, and stirred for 30 min. The resulting solid was filtered and washed with methanol and sodium bicarbonate to remove unreacted acid and allowed to dry

overnight to give the desired product as a brown solid (5.8 g). The product was characterized by electrospray mass spectroscopy (mp >300° C).

2.(4-Aminophenyl)-5-aminobenzimidazole was prepared by suspending the above solid (75 g) in THF (75 ml), to which was added Pd-C (10% Pd by weight). The flask was purged with hydrogen and stirred under a balloon of hydrogen over night. TLC and MS showed starting material was still present so the reaction was allowed to continue over the weekend. TLC indicated complete reaction, the reaction was filtered through celite and washed with methanol. The solvent was removed under reduced pressure to give a dark brown solid (0.37 g) that was used without further purification.

Alternatively, the 2-(4-aminophenyl)-5-aminobenzimidazole was prepared by the following reduction: 2-(4-nitrophenyl)-6-nitrobenzimidazole (8.9 g, 31 mmole) was suspended in concentrated HCl (100 ml) to which was added stannous chloride (42.3 g 180 mmole). The reaction mixture was heated to reflux for 5 hrs. The mixture was cooled to RT and the HCl salt of the desired product was precipitated by the addition of ethanol. The resulting solid was filtered, re-dissolved in water and the solution made basic by the addition of concentrated ammonium hydroxide. The resulting precipitate was filtered and dried overnight under vacuum to yield the desired product as a gray solid (6.023 g, 26.9 mmole, 87%). The product characterized by electrospray mass spectroscopy and HPLC (mp. 222-227° C).

2.(4-Aminophenyl)-5-methoxy benzimidazole was synthesized from 2-(4-nitrophenyl)-5-methoxy benzimidazole, which was prepared as follows: 1,2-diamino-4-methoxybenzene (1.26 g, 10.0 mmole was mixed with 4-nitrobenzoic acid (1.67 g, 9.8 mmole) and dissolved in $POCl_3$ (10 ml) and heated to reflux for 2.5 hours. The reaction mixture was cooled and cautiously poured onto ice. The resulting solid was filtered, washed with $NaHCO_3$ and used without further purification.

2.(4-Aminophenyl)-5-methoxy benzimidazole was prepared by dissolving 1 g of the above nitrobenzimidazole in 30% $Na_2S{\cdot}9H_2O$ (20 ml) with stirring at RT for 21 h. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic extracts were dried over sodium sulfate and concentrated under vacuum. The product was characterized by mass spectroscopy.

2.(4-Aminophenyl)-5,6-dichloro benzimidazole was synthesized from 2-(4-nitrophenyl)-5,6-dichloro benzimidazole, which was prepared as follows: 1,2-diamino-4,5-dichlorobenzene (1.68 g, 10.0 mmole) was mixed with 4-nitrobenzoic acid (1.58 g, 9.3 mmole), dissolved in $POCl_3$ (10 ml), and heated to reflux for 2.5 hours. The reaction mixture was cooled and cautiously poured onto ice. The resulting solid was filtered, washed with $NaHCO_3$ and used without further purification.

2.(4-Aminophenyl)-5,6-dichloro benzimidazole was prepared by dissolving 1 g of the above nitrobenzimidazole in 30% $Na_2S{\cdot}9H_2O$ (20 ml) with stirring at RT for 21 h. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic extracts were dried over sodium sulfate and concentrated under vacuum. The product was characterized by mass spectroscopy.

2.(4-aminophenyl)-7-methyl benzimidazole was synthesized from 2-(4-nitrophenyl)-7-methyl benzimidazole, which was prepared by mixing 1,2-diamino-3-methylbenzene (1.24 g, 10.0 mmole) with 4-nitrobenzoic acid (1.69 g, 9.8 mmole), dissolved in $POCl_3$ (10 ml), and heated to reflux for 2.5 hours. The reaction mixture was cooled

and cautiously poured onto ice. The resulting solid was filtered, washed with $NaHCO_3$ and used without further purification.

2.(4-Aminophenyl)-7-methyl benzimidazole was synthesized by dissolving 1 g of the above nitrobenzimidazole in 30% $Na_2S \cdot 9H_2O$ (20 ml) with stirring at RT for 4.5 h. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic extracts were dried over sodium sulfate and concentrated under vacuum. The product was characterized by mass spectroscopy.

2.(4-Aminophenyl)-6-methyl benzimidazole was synthesized from 2-(4-nitrophenyl)-6-methyl benzimidazole, which was prepared by mixing 1,2-diamino-4-methylbenzene (1.24 g, 9.8 mmole) with 4-nitrobenzoic acid (1.6 g, 9.9 mmole) and dissolved in $POCl_3$ (10 ml) and heated to reflux for 2.5 hours. The reaction mixture was cooled and cautiously poured onto ice. The resulting solid was filtered, washed with $NaHCO_3$ and used without further purification.

2.(4-Aminophenyl)-6-methyl benzimidazole was synthesized by dissolving 1 g of the above nitrobenzimidazole in 30% $Na_2S \cdot 9H_2O$ (20 ml) with stirring at RT for 4.5 h. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic extracts were dried over sodium sulfate and concentrated under vacuum. The product was characterized by mass spectroscopy.

2.(4-Aminophenyl)-5,6-dimethyl benzimidazole was synthesized from 2-(4-nitrophenyl)-5,6-dimethyl benzimidazole, which was prepared by mixing 1,2-diamino-4,5-dimethylbenzene (1.38 g, 10.1 mmole) with 4-nitrobenzoic acid (1.69 g, 9.9 mmole) and dissolved in $POCl_3$ (10 ml) and heated to reflux for 2.5 hours. The reaction mixture was cooled and cautiously poured onto ice. The resulting solid was filtered, washed with $NaHCO_3$ and used without further purification.

2.(4-Aminophenyl)-5,6-dimethyl benzimidazole was synthesized by dissolving 1 g of the above nitrobenzimidazole (31.1) in 30% $Na_2S \cdot 9H_2O$ (20 ml) with stirring at RT for 4.5 h. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic extracts were dried over sodium sulfate and concentrated under vacuum. The product was characterized by mass spectroscopy.

The subsequent preparation of symmetrical diamides was accomplished by one of the following methods:

Method A: 2-(4-Aminophenyl)-6-aminobenzimidazole (1 mmole) was suspended in THF (5 ml) to which was added DIEA (2.5 mmole) and mixture cooled to -78° C. To the above cooled mixture was added the acid chloride (2.5 mmole) and let warm to RT overnight. Water (2 ml) is added to the reaction and extracted with EtOAc. The combined organic extracts were combined washed with $NaHCO_3$ (aq.) and concentrated under reduced pressure. The resulting residue was purified on silica gel (hexanes/EtOAc or $MeOH/CH_2Cl_2$) or reverse phase HPLC (CH_3CN/H_2O).

Method B: 2-(4-Aminophenyl)-6-aminobenzimidazole (1 mmole) and DMAP (cat.) was dissolved in pyridine (5 ml). To the above solution was added the acid chloride (2.5 mmole) and the reaction stirred overnight at 60° C. The reaction was cooled to room temperature and water added to precipitate the product. The resulting solid was collected by filtration with the solid being washed by hexanes and water and $NaHCO_3$

(aq.). The resulting residue was purified on silica gel (hexanes/EtOAc or MeOH/CH_2Cl_2) or reverse phase HPLC (CH_3CN/H_2O).

Method C: 2-(4-Aminophenyl)-6-aminobenzimidazole (1 mmole) was suspended in THF (10 ml) to which was added K_2CO_3 (2.5 mmole) in water (0.5 ml). and mixture cooled to -78° C. To the above cooled mixture was added the acid chloride (2.5 mmole) and let warm to RT overnight. Water (10 ml) was added to the reaction and extracted with EtOAc. The combined organic extracts were combined washed with $NaHCO_3$ (aq.) and concentrated under reduced pressure. The resulting residue was purified on silica gel (hexanes/EtOAc or MeOH/CH_2Cl_2) or reverse phase HPLC (CH_3CN/H_2O).

Method D: The carboxylic acid (2.2 mmole), EDC (2.2 mmole) and DMAP (cat.) was dissolved in hot pyridine. To the above solution was added 2-(4-aminophenyl)-6-aminobenzimidazole (1 mmole) and heated to 60° C overnight. The cooled reaction mixture was partitioned between water and EtOAc. The organic layer was washed with $NaHCO_3$, dried over Na_2SO_4 and concentrated under vacuum. The resulting residue was purified on silica gel (hexanes/EtOAc or MeOH/CH_2Cl_2) or reverse phase HPLC (CH_3CN/H_2O).

Diacyl Benzimidazole Species

The following species encompassed within the disclosed generic formula were synthesized and tested for their ability to suppress IgE. The species are presented above in the Summary of the Invention.

Suppression of IgE Response

The inhibitory activity of the small molecules of the present invention were assayed using both the *ex vivo* and *in vivo* assays as described above. All of the compounds presented above were active in suppressing the IgE response. In the *ex vivo* assay, compounds in genuses I-XI produced 50% inhibition at concentrations ranging from 1 pM to 10 μM. In the *in vivo* assay, the compounds were effective at concentrations ranging from less than about 0.01 mg/kg/day to about 25 mg/kg/day, when administered in divided doses (e.g., two to four times daily) for at least two to seven consecutive days. Thus, the small molecule inhibitors of the present invention are disclosed as being useful in lowering the antigen-induced increase in IgE concentration, and consequently, in the treatment of IgE-dependent processes such as allergies in general and allergic asthma in particular.

Treatment Regimens

The amount of the IgE inhibitor compound which may be effective in treating a particular allergy or condition will depend on the nature of the disorder, and can be determined by standard clinical techniques. The precise dose to be employed in a given situation will also depend on the choice of compound and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Appropriate

dosages can be determined and adjusted by the practitioner based on dose response relationships between the patient's IgE levels as well as standard indices of pulmonary and hemodynamic changes. Moreover, those skilled in the art will appreciate that dose ranges can be determined without undue experimentation by following the protocol(s) disclosed herein for *ex vivo* and *in vivo* screening (See for example Hasegawa et al., *J. Med. Chem.* 40: 395-407 (1997) and Ohmori et al., *Int. J. Immunopharmacol.* 15:573-579 (1993); employing similar *ex vivo* and *in vivo* assays for determining dose-response relationships for IgE suppression by naphthalene derivatives; incorporated herein by reference).

Initially, suitable dosages of the compounds will generally range from about 0.001 mg to about 300 mg per kg body weight per day in divided doses, more preferably, between about 0.01 mg and 100 mg per kg body weight per day in divided doses. The compounds are preferably administered systemically as pharmaceutical formulations appropriate to such routes as oral, aerosol, intravenous, subcutaneously, or by any other route which may be effective in providing systemic dosing of the active compound. The compositions of pharmaceutical formulations are well known in the art. The treatment regimen preferably involves periodic administration. Moreover, long-term therapy may be indicated where allergic reactions appear to be triggered by continuous exposure to the allergen(s). Daily or twice daily administration has been effective in suppressing the IgE response to a single antigen challenge in animals when carried out continuously from a period of two to seven consecutive days. Thus, in a preferred embodiment, the compound is administered for at least two consecutive days at regular periodic intervals. However, the treatment regimen, including frequency of dosing and duration of treatment may be determined by the skilled practitioner, and modified as needed to provide optimal IgE down-regulation, depending on nature of the allergen, the dose, frequency, and duration of the allergen exposure, and the standard clinical indices.

In one embodiment of the present invention, an IgE-suppressing compound may be administered in conjunction with one or more of the other small molecule inhibitors disclosed, in order to produce optimal down-regulation of the patient's IgE response. Further, it is envisioned that one or more of the compounds of the present invention may be administered in combination with other drugs already known or later discovered for treatment of the underlying cause as well as the acute symptoms of allergy or asthma. Such combination therapies envisioned within the scope of the present invention include mixing of one or more of the small molecule IgE-inhibitors together with one or more additional ingredients, known to be effective in reducing at least one symptom of the disease condition. In a variation, the small molecule IgE-inhibitors herein disclosed may be administered separately from the additional drugs, but during the same course of the disease condition, wherein both the IgE-inhibitor(s) and the palliative compounds are administered in accordance with their independent effective treatment regimens.

While a number of preferred embodiments of the invention and variations thereof have been described in detail, other modifications and methods of use will be readily apparent to those of skill in the art. Accordingly, it should be understood that various applications, modifications and substitutions may be made of equivalents without departing from the spirit of the invention or the scope of the claims.

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